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THE ACCURACY OF THE PLATE COUNT OF SUSPENSIONS OF PURE CULTURES OF BACTERIA IN STERILE SOIL[1]

BY MARJORIE SUTHERLAND[2] AND NORMAN JAMES[3]

Abstract

A culture of *Pseudomonas fluorescens* was suspended in a sterile soil and water mixture. Dilutions of 1 : 2,000,000 and 1 : 10,000,000 were made immediately and plated in four replicates of each dilution, using nutrient agar. This was repeated 200 times. A χ^2 value was calculated from each set of four counts. The distribution of the 200 χ^2 values in the platings from each dilution agrees very well with the theoretical distribution. In a second experiment, 100 sets of four replicates of *Pseudomonas fluorescens* were plated along with 100 of *Bacterium globiforme* and 100 of a mixture of the two cultures. The distribution of the χ^2 values in each of the three sets is such that the values may be considered to have been derived from populations distributed according to the Poisson series.

The close conformity of the distribution of the actual χ^2 values to that of the expected in each of the five sets of data appears to indicate that the mean of four replicates is reliable as an estimate of the population in the dilution plated; and further, that the failure to obtain this conformity with soil flora is due to other causes than technique.

Introduction and Historical

In soil microbiology, some practical method of obtaining estimates of the individual populations in soils of various types is needed. The accurate estimation of numbers of micro-organisms may be of value in the measurement of soil potentialities or the effect of soil treatment. Present available methods are used with little knowledge of their accuracy. There is not general agreement on a test to determine how much of the variation among replicates prepared from a single sample is due to random sampling and how much is due to characters inherent in the population.

The plate method has been the most popular means of counting organisms in soil or elsewhere since Koch's development of the liquefiable solid medium in 1881. Certain limitations of the method are recognized. The result obtained does not represent a count of the actual numbers in the original sample but only of such organisms or clumps of organisms as grow to form colonies under the conditions provided. Many investigators have found numerous sources of error in the plating technique. In spite of the shortcomings of the method, a large part of the advance made in bacteriology is based

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upon plate counts. Using milk and cultures of the colon organism, Breed and Stocking (1) found that skilled analysts with proper technique usually make reasonably accurate estimates of the number of living bacteria in milk. More experience and improved practices result in more regular and supposedly more accurate counts.

One of the more recent approaches in testing the value of bacteriological data is based upon statistical methods. Fisher, Thornton and MacKenzie (4) suggested the χ^2 (Chi square) test for use on large numbers of counts of parallel plate series. The object of this test is to determine whether the variation found in a series of replicate plates is due to random sampling. Harmsen and Verweel (6) give a brief summary of the reasoning involved in the development and use of the χ^2 test.

In the test, a value of χ^2 appropriate to the Poisson distribution is worked out for each set of parallel plates by the equation

$$\chi^2 = \frac{S(x-\bar{x})^2}{\bar{x}}$$

where x is the number of colonies counted on a plate and $\bar{x}$ is the mean of the set. Fisher's table of χ^2 gives the probability of the occurrence of given χ^2 values in an infinite population for selected degrees of freedom. The P values range from .99 to .01. The class boundaries are the χ^2 values for these selected values of P. On the basis of one hundred samples, there should be one χ^2 value with a P greater than .99, one between .99 and .98, three between .98 and .95, five between .95 and .90, etc., until finally one with a χ^2 value less than .01. The χ^2 values for class boundaries are chosen for degrees of freedom equal to one less than the number of plates in the series. The observed values are placed in their respective classes and the actual compared with the theoretical or expected distribution.

A goodness-of-fit test (3) is used to ascertain the extent of this agreement. The observed and theoretical values for each class in the distribution are set up. From the formula $\frac{(actual—theoretical)^2}{theoretical}$ a value is calculated for each class. These are totalled and the probability of the occurrence of the final χ^2 value, obtained in this manner, is determined by finding the corresponding P value. A final χ^2 value with a P of .50 is accepted as indicating a perfect fit. A close agreement with the theoretical is accepted as indicating that the means of the series are reliable, and that the data give no reason for questioning the hypothesis tested. Fisher, Thornton and MacKenzie (4) believe that close agreement with the theoretical distribution is rare but possible, and that the conditions may be satisfied with simple flora or certain mixtures of organisms.

Harmsen and Verweel (6) carried out experiments with soil platings, making parallel sets of ten each. They state that their results for the total counts of bacteria and actinomycetes show too many high χ^2 values. Technique was eliminated as the cause, since their results with starch-disintegrating

organisms, protein-disintegrating organisms, and actinomycetes alone show reasonably good conformity with the theoretical distribution. Next, they grouped their results on the basis of numbers on the plates and tested the χ^2 distribution for these sets. An equally bad distribution resulted. As a last resort, this test was applied to similar data published by Waksman (12) in 1920–21, which was found to give a similar distribution when submitted to the χ^2 test. Work done in 1936 in the Bacteriology Laboratory of the University of Manitoba on a large number of field samples confirms the finding referred to above, namely, that there is some factor, other than random sampling, responsible for the variation among counts of bacteria obtained from a series of replicate platings of one dilution of field soil. A report on this and additional data from studies in 1937 will appear at an early date.

Wilson and Kullmann (13) sought to overcome this difficulty, found in plating pure cultures of rhizobia also, by discarding a χ^2 value over ten, if the variation was due chiefly to one plate. In this way they obtained a very good fit. Another method used by the same investigators was to pour a set of five plates and eliminate one, resulting in a four plate set. Any plate showing marked deviation from the other four was eliminated, otherwise, the third plate was discarded arbitrarily. It is difficult to understand how this can be done without bias.

Scope of Problem

From an examination of the literature it is evident that, before the means of replicate sets of bacterial counts may be used with confidence, it is necessary to determine the cause of the too frequent occurrence of high χ^2 values in the reports referred to above. Therefore, three points were selected for study.

1. It appeared proper to consider first whether the method of making dilutions and preparing plates, as used in this laboratory, gives reliable results when a pure culture of bacteria is used.

2. At the same time it seemed desirable to determine whether there is a change in the distribution of χ^2 values when dilutions yielding high or low counts are used.

3. The effect of mixing two pure cultures of common soil organisms was suggested as the next logical procedure.

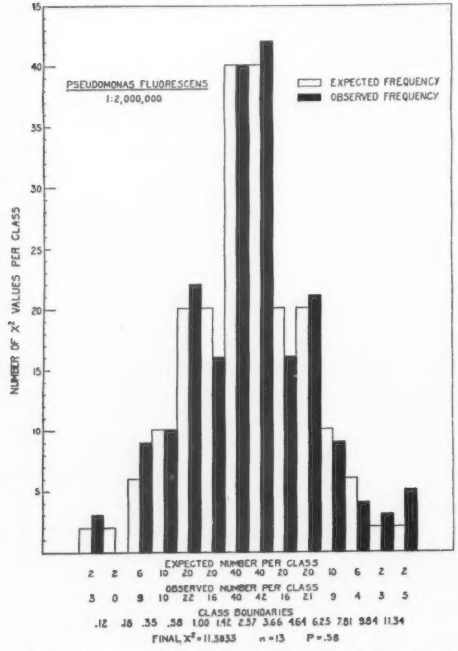
Experiment 1

This experiment was designed to examine the first and second points. It is known that non-spore-forming bacteria are more representative of the general types found in soil than the spore formers. Waksman (9) lists the common heterotrophic non-spore-formers as "*Bact. fluorescens*, *Bact. caudatum*, *Bact. radiobacter*, etc." *Pseudomonas fluorescens* was chosen as the test organism for this study. A stock culture was used. The culture was grown on agar slants for one to three days at 26° C. This time variation was used in order that the counts would not be influenced by growth phase phenomena.

In preparing the first dilution, sterile soil was added in an attempt to duplicate the effect of shaking the natural soil sample. There may be some physical or chemical condition in soil, not usually found in pure cultures from agar slants, that affects the count obtained. The dilution blanks were prepared so that there was approximately 245 ml. of water in the first and 99 ml. in the others, after sterilizing.

To prepare one set of plates, a streak culture of *Pseudomonas fluorescens* was flooded with 5 ml. of sterile water, the growth scraped from the agar and the suspension added to a 245 ml. blank, after which 25 gm. of sterile soil was introduced. This dilution was shaken for 5 min. in an automatic shaker, and dilutions were made to 1 : 2,000,000 and 1 : 10,000,000. Four plates were made from each final dilution, using one pipette for each dilution. About 10 ml. of standard nutrient agar was added to each plate.

Nutrient agar was used for plating because of its general nature. Since *Pseudomonas fluorescens* normally is not a spreading type there was little need for a medium designed to control spreaders. Bottom spreaders developed sometimes when the agar was not added immediately after the delivery of the sample to the plate. When this occurred the entire set of four plates from each dilution of the sample was discarded, as it was impossible to secure an accurate count or estimate of the original number of organisms. Contamination was considered a reason for discarding sets of plates, but excessive variation in numbers of colonies was not. The plates were incubated for three to five days, the colonies counted, and the counts checked with the hand lens. At three days, some of the colonies were missed with the unaided eye, but were found when checked with the hand lens. An increase in the number of colonies was not noted when the longer period of incubation was used, but the count was made more readily at the end of the five-day period.



HISTOGRAM 1. *χ^2 distribution on 200 samples for the 1 : 2,000,000 dilution of Pseudomonas fluorescens.*

Counts of 200 sets of four replicates of the 1 : 2,000,000 dilutions were obtained by this procedure, and another

200 sets on the 1 : 10,000,000 dilutions of the same samples. The χ^2 value for each set of counts of four replicates was calculated. These values were then distributed in their respective classes on the basis of class boundaries for three degrees of freedom, and the actual was compared with the theoretical distribution. Finally, the goodness-of-fit test, using 13 degrees of freedom, was applied to ascertain the extent of the agreement between the actual and theoretical distributions.

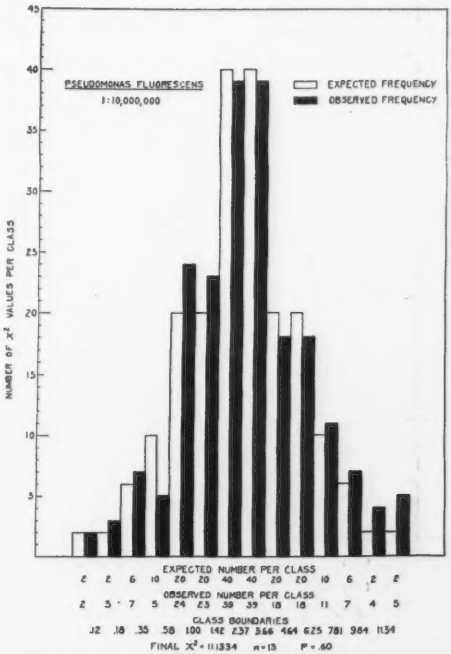
Results of Experiment 1

The actual counts, calculated means, and χ^2 values are available (7) but are not included. A summary of the actual and theoretical distributions of the 200 χ^2 values, at 1 : 2,000,000 dilution, is presented in Histogram 1, and at 1 : 10,000,000 dilution, in Histogram 2. In each case the agreement appears close. The goodness-of-fit test gives a final χ^2 value of 11.3833 and a P value of .58 at 1 : 2,000,000 dilution; and 11.1334 and .60 respectively at 1 : 10,000,000 dilution.

Experiment 2

The second experiment deals with the effect of mixing *Pseudomonas fluorescens* with a pure culture of another common soil organism. Taylor and Lochhead (8), in following up work done by Conn and Darrow (2), reported that organisms of the type of *Bacterium globiforme* represent some 10% of the organisms capable of being isolated by the plate method. This type of organism grows rapidly on nutrient agar. Dr. Lochhead very kindly provided a culture of *Bacterium globiforme* N. G. 53.

A dilution of 1 : 10,000,000 was used, since the low counts have been shown to give an equally good χ^2 distribution and involve less work. In order to have results on the separate cultures used in the mixture, sets of plates of *Pseudomonas fluorescens* and of *Bacterium globiforme* were made at the same time and from the same dilutions as used in preparing the mixtures. It was thought that

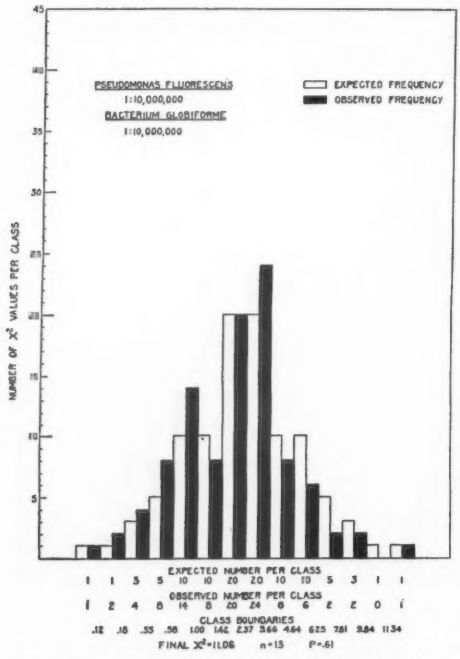


HISTOGRAM 2. *χ^2 distribution on 200 samples for the 1 : 10,000,000 dilution of Pseudomonas fluorescens.*

these might throw some light on the cause of the variation. One pipette was used for each dilution of each culture. One ml. of the final dilution of *Pseudomonas fluorescens* was placed in each of eight plates. One ml. aliquots of the final dilution of *Bacterium globiforme* were added to four of these and to four other plates. This resulted in a set of four plates each of *Pseudomonas fluorescens*, *Bacterium globiforme*, and a mixture of the two. One hundred sets of twelve plates were prepared by this procedure. The data in each set of four replicates were submitted to the mathematical treatment referred to under Experiment 1.

Results of Experiment 2

In this experiment also the actual counts and calculated values are not included; nor are the summaries of the counts on the separate sets with the pure cultures. These are available (7) and show essentially the same result as is presented in Experiment 1. The goodness-of-fit test on the distribution of the 100 samples of *Pseudomonas fluorescens* gives a final χ^2 value of 12.13 and a P value of .52; and in the 100 samples of *Bacterium globiforme* of 7.90 and ·54 respectively. In the case of the latter culture these final values were obtained by piling (3, 5) the small classes at the extremities of the histogram, thus reducing the number of classes to ten and the degrees of freedom to nine.



Histogram 3. *χ^2 distribution on 100 samples for a mixture of the 1 : 10,000,000 dilutions of Pseudomonas fluorescens and Bacterium globiforme.*

The distribution of the 100 χ^2 values, calculated from the data obtained from the plates with the two species of bacteria, is shown in Histogram 3, and represents a close agreement with what would be expected from random sampling of a population distributed according to the Poisson series. The goodness-of-fit test gives a final χ^2 value of 11.06 and a corresponding P value of .61.

Discussion of Results

The goodness-of-fit test applied to the χ^2 distribution involves an acceptance of a P value within certain definite limits. Fisher (3) states that a

range of values from .90 to .10 may be expected without questioning the hypothesis tested. A P value outside these limits may be taken as indicating that the hypothesis tested does not account for all the factors involved. In the distributions reported herein, the P values are well within and indeed are close to the mid-point of these two limits. Consequently, one may assume that the variations among counts obtained in these studies are the result of random sampling, rather than of serious error in the technique of plating and counting. The mean of four replicate plates of certain pure cultures, or mixtures of them, may be accepted as providing a reasonable estimate of the population sampled. This confirms the opinion expressed by Fisher, Thornton and MacKenzie (4), referred to in the introduction.

The finding of an equally good fit in the χ^2 distributions in the two dilutions reported under Experiment 1 is of interest from the standpoint of results obtained with field soils. The failure to obtain a good fit, when considering the total count of bacteria in the soil by the plate method, may be due to certain associative actions, antagonistic or stimulative, among organisms in the plate. The question of associative and antagonistic effects of microorganisms has been reviewed fully by Waksman (10). A high dilution means a small number of organisms in a given plate, and consequently less chance of the presence of certain antagonistic types and also less associative action because of the greater distance between organisms on the plate. However, since the variance in counts from each dilution conforms to expectation in the Poisson series, the standard error of the mean of four small-count replicates is greater than that of four large counts. In plating field soils, compensation for this loss in accuracy could be obtained by increasing either the number of plates from one dilution or the number of replicate samples plated (11).

The finding of Experiment 2 provides no indication of a disturbing factor when these two species appear in a plate. This is suggested by the χ^2 test, and by the fact that the average of the 100 sets of the mixture is 126 colonies per plate, while the sums of the separate platings average 125. Of course, this may not hold for other species of bacteria.

The results of these two studies have a definite bearing on platings from soil. Since the routine of making dilutions and plating was the same as is used with field samples of soil, the factor of laboratory technique may be eliminated as a cause of the discrepancies in the χ^2 distributions, as observed in our laboratory and reported by other investigators.

Conclusions

1. The plating technique used in this study produces sets of four replicate counts of certain pure cultures of bacteria, or mixtures of them, whose χ^2 values are distributed according to the Poisson series. The technique of diluting, plating and counting is the same as that used in this laboratory with soil samples handled on a large scale.

2. Dilutions yielding 25 to 75 colonies per plate give as good a χ^2 distribution as those from the same samples yielding five times as many colonies per plate.

3. The failure to obtain agreement between the actual and theoretical distributions of χ^2 values for counts of bacteria from soil samples is the result of factors other than the technique used in these investigations.

Acknowledgments

The writers are pleased to express their appreciation to Dr. C. H. Goulden, of the Dominion Rust Research Laboratory, Winnipeg, for advice on the statistical aspects of this investigation, and to Mr. A. M. Brown, of the same laboratory, for photographing the histograms.

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EFFECT OF PHYTOHORMONES ON SEEDS DAMAGED BY FORMALDEHYDE AND OTHER DISINFECTANTS[1]

By N. H. Grace[2]

Abstract

Experiments with cereal seeds demonstrate that the reduction in germination and early growth resulting from formaldehyde treatment can be largely overcome by adding the phytohormones, 1-naphthylacetic acid or 3-indolylacetic acid, to the disinfecting solution. The optimum concentration of the hormone for individual varieties of cereals lies between 0.01 and 5 p.p.m. Similar effects were also obtained with hormones after copper sulphate and hot water treatments. The method appears to have practical possibilities, and may also be useful for comparing the physiological activities of different compounds.

Introduction

Seed treatment for the prevention of certain diseases of cereals has been practised for several centuries (10), but the advantages which result are frequently offset by the inhibiting effect of the disinfectant on germination and subsequent growth. Such effects are particularly serious with the widely used formaldehyde treatment for smut control. Seed injury occurs under most conditions and is increased when seeding is delayed after treatment, when the soil is dry, or when low-grade seed is used. As a result, this treatment is being gradually displaced by others such as those involving the use of copper carbonate and organic mercurial dust disinfectants.

Various hypotheses have been put forward to explain seed injury by disinfectants, and methods have been suggested for its reduction (1–3, 8, 9, 11). Amongst these hypotheses, that of Henry (7) seemed particularly interesting. Using the oat coleoptile as an indicator, he demonstrated that formaldehyde tends to inactivate the growth hormone, heteroauxin, and he suggested that this inactivation might account, in part, for the reduction in germination and growth caused by the disinfectant. It occurred to the writer that this hypothesis might be tested by adding a growth-promoting chemical to formaldehyde solutions used for treating seeds. If inactivation of heteroauxin is the main factor, addition of a chemical which is less sensitive to oxidation might reduce injury. Moreover, it seemed possible that such investigations might lead to the development of an improved solution method for seed treatment.

Experiments have been made with the two physiologically active chemicals, 1-napthylacetic and 3-indolylacetic acids, using a number of different types of cereal seed, and formaldehyde, copper sulphate, and hot water seed treatments.* The results demonstrate that hormones reduce the deleterious effects of the disinfectants very considerably. The method also appears to have possibilities for the comparison of the physiological activities of different chemicals.

[1] *Manuscript received July 21, 1938.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa.

[2] *Chemist, National Research Laboratories, Ottawa.*

* *A preliminary report appeared in Nature, 142 : 77. 1938.*

Methods

Treatment

Cereal seeds were treated by sprinkling 25 gm. of seed with 5 cc. of a solution of 1 : 320 commercial formaldehyde (37% by weight of the gas) in water. This method was found to be more convenient than that of immersing the seeds in the solution for 10 min. The latter method was used in some earlier experiments, as noted later. After treatment, the samples were placed on filter paper and covered with inverted cans for 4 hr. They were then loosely wrapped in small pieces of canvas to prevent aeration and provide conditions favorable to formaldehyde damage. When planting occurred more than one day after treatment, the seed was stored in open cans to permit continued loss of moisture.

The copper sulphate treatment consisted of soaking seed for 5 min. in a solution containing 12 gm. of $CuSO_4 . 5H_2O$ in 500 cc. of water, draining, and drying for 24 hr. before planting. Hot-water disinfection involved presoaking the wheat for 4 hr. at 30° C., then placing the samples in solutions for 10 min. in a bath maintained at 54° C. The seed was spread in a thin layer on filter paper to cool and dry for 24 hr. before use.

Freshly prepared solutions were employed in treatment, except in a few experiments where the stability of the chemical was under test. The hormone* chemical was introduced in the treating solution and the concentration expressed in p.p.m. (parts per million) by weight.

Estimation of Effect of Treatments

Seeds were spread on wet blotting paper in 50- or 100-kernel lots and placed in a germinator at 18° C. Germination counts were made 7 to 10 days after planting.

In most of the investigations, 50 seeds were also planted in soil in small cardboard flats and kept in the greenhouse at about 16° to 20° C. After 15 to 22 days, all plants grown from the 50 seeds were collected and washed. Germination counts were made at this time. A number of plants (10, 15, 20 or 30, depending on the time and help available) were then selected at random and measured. The stem was measured from the seed to the tip of the longest leaf, and the sum of the lengths of all seminal roots was also determined. The data are recorded as mean lengths for single plants. All of the plants from 50 seeds were then dried for 4 hr. in an oven at 93° C., conditioned for one week in the laboratory, and weighed. The data are recorded as air-dry weight of plants from 50 seeds.

Experiments with Formaldehyde and Formaldehyde-hormone Treatments

Effects on Plants Grown in Soil

Data on germination, dry weights, and root and stem measurements for five pure varieties of wheat and two samples of low commercial grades are

* *For the sake of convenience, the physiologically active chemicals used in the treatments have been designated by the term "plant hormone". It is recognized that there is some question as to the accuracy of this term.*

given in Tables I and II. The low grade wheats were included since, in poor crop years, such wheats are sometimes used for seed and are particularly subject to formaldehyde damage.

TABLE I

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON GERMINATION AND DRY WEIGHT OF WHEAT PLANTED IN SOIL

Treatment	Hormone concentration, p.p.m.	Germination, %						
		Varieties					Commercial wheats	
		Garnet	Marquis	Mindum	Red Fife	Reward	No. 5 wheat, 1935	No. 6 Special wheat, 1935
Untreated control		99	98	82	97	95	82	94
Formaldehyde control		74	95	63	84	65	30	82
Formaldehyde and naphthylacetic acid	0.01	74	97	80	86	94	–	92
	0.1	87	91	77	97	92	70	–
	1.0	80	96	75	94	93	78	86
	1.67	–	–	–	–	–	66	–
	3.0	75	94	83	98	88	–	80
Formaldehyde and indolylacetic acid	0.01	75	94	69	–	91	–	–
	0.1	80	94	82	–	92	74	–
	1.0	92	94	72	–	96	88	–
	1.67	–	–	–	–	–	60	–
	3.0	95	89	67	–	90	–	–
Necessary difference, 5% level		20	20	20	6	20	–	–
		Dry weight of plants from 50 seeds, gm.						
Untreated control		0.89	1.01	1.05	0.64	1.14	–	0.52
Formaldehyde-treated control		0.47	0.93	0.74	0.46	0.57	–	0.43
Formaldehyde and naphthylacetic acid treatment	0.01	0.61	1.01	1.05	0.54	1.01	–	0.46
	0.1	0.71	0.89	1.12	0.59	0.96	–	–
	1.0	0.60	0.90	0.92	0.64	0.90	–	0.43
	1.67							
	3.0	0.60	0.93	1.09	0.67	0.86	–	0.48
Formaldehyde and indolylacetic acid treatment	0.01	0.65	0.85	0.93	–	1.05	–	–
	0.1	0.64	0.94	1.10	–	1.01	–	–
	1.0	0.83	0.93	0.99	–	0.95	–	–
	1.67	–	–	–	–	–	–	–
	3.0	0.81	0.85	0.89	–	1.02	–	–
Necessary difference, 5% level		0.29	0.29	0.29	0.05	0.29	–	–

Germination data are given in the upper half of Table I as means of duplicate counts of 50 seeds. Formaldehyde damaged all samples except Marquis, and it is apparent that when damage occurred, both hormones tended to reduce it. Although the variation between duplicate counts was large, statistical

analysis shows that with Garnet, Mindum, Red Fife and Reward, a concentration of hormone was reached which resulted in a significant improvement in germination, when compared with the formaldehyde control. The commercial samples were not replicated but, though no statistical treatment is possible, the results obviously show the same trends.

TABLE II

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON LENGTH OF ROOTS AND STEMS OF WHEAT PLANTS GROWN IN SOIL

Variety	Treatment	Roots, mm.	Stems, mm.
Mindum	Untreated control	392	179
	Formaldehyde control	202	170
	Formaldehyde and 0.01 p.p.m. naphthylacetic acid	488	175
	Formaldehyde and 1.0 p.p.m. indolylacetic acid	552	207
Marquis	Formaldehyde control	337	156
	Formaldehyde and 0.1 p.p.m. naphthylacetic acid	374	174
	Formaldehyde and 0.1 p.p.m. indolylacetic acid	403	163
Garnet	Formaldehyde control	158	141
	Formaldehyde and 1.0 p.p.m. naphthylacetic acid	393	176
Necessary difference, 5% level		65	10

Data on the dry weight of plants from 50 seeds are given in the lower half of Table I. The dry weight of all the pure varieties, except Marquis, is significantly reduced by formaldehyde treatment. There is a statistically significant increase above the formaldehyde control in every formaldehyde-hormone treatment of Red Fife and Reward. Garnet shows a significant increase at the one and three p.p.m. levels of indolylacetic acid, while Mindum gives the most uniform response with naphthylacetic acid. Several of the formaldehyde-hormone treatments of the four pure varieties give dry weights which are not significantly inferior to that of the untreated control. The data leave little doubt that reduction of formaldehyde damage is effected by the use of formaldehyde-hormone treatments on both high and low grades of seed.

Data on mean root and stem measurements of ten plants, selected at random, are given in Table II. Formaldehyde damage to Mindum is shown by a significant decrease in the roots. Root length is increased by the addition of 0.01 p.p.m. naphthylacetic or 1 p.p.m. indolylacetic acids, so that it becomes greater than that of the untreated control. The stem length at 1 p.p.m. indolylacetic is also significantly improved. Marquis shows a significant improvement in stems with 0.1 p.p.m. naphthylacetic acid, and a significant increase in roots with 0.1 indolylacetic acid. It is not surprising that the improvement is slight, as germination and dry weight data on Marquis indicated little damage. Garnet, however, shows a striking increase in both roots and stems as a result of the addition of hormone. The data strongly

suggest that prevention of formaldehyde damage is associated with the increased root development following the addition of hormones to the treating solutions.

Effect of Using Greater Concentrations of Hormones

Data on germination and dry weights of Red Fife wheat and Banner oats are given in Table III. The weights are for the plants grown from 50 seeds and the germination results are from both germinator and soil studies. In the range between one and five p.p.m. of naphthylacetic acid, an optimum is reached. The dry weight of wheat is significantly better than that of the untreated control at five p.p.m., and oats are better at three p.p.m. Not only has damage been completely avoided but a net stimulation occurs. A significant drop is observed at 10 and 15 p.p.m., indicating the overdosage phenomenon characteristic of plant response to these active chemicals.

TABLE III

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE PLUS HIGHER CONCENTRATIONS OF HORMONE ON GERMINATION AND DRY WEIGHT OF WHEAT AND OATS

Treatment	Hormone concentration, p.p.m.	Germination, %				Dry weight of plants from 50 seeds grown in soil, gm.	
		Germinator		Soil			
		Red Fife wheat	Banner oats	Red Fife wheat	Banner oats	Red Fife wheat	Banner oats
Untreated control		95	93	97	99	0.64	0.93
Formaldehyde control		83	91*	84	78	0.46	0.62
Formaldehyde and naphthylacetic acid	0.01	95		86		0.54	
	0.1	98	95	97	93	0.59	0.83
	1	98	98	94	94	0.64	0.82
	3	98	93	98	100	0.67	1.10
	5	99	93	92	87	0.69	0.83
	10	95	97	93	84	0.59	0.78
	15	89		92		0.55	
Necessary difference, 5% level		6	4	6	15	0.05	0.16

* *The wheat was treated with the usual 1 : 320 formaldehyde solution, but the oats with 1 : 180, as the higher concentration was required to show damage.*

Comparison of Commercial Formaldehyde and Polymer-free Formaldehyde

It has been suggested that formaldehyde damage is associated with a layer of paraformaldehyde which forms on the surface of the treated seed during drying. Earlier work in this laboratory showed that polymer-free formaldehyde caused essentially the same damage as the commercial product which contains some polymer, and that substantial increase in the methyl alcohol content had little effect. While it appeared unlikely that the presence of polymer would seriously affect the extent of damage, this aspect has received consideration. A sample of polymer-free formaldehyde* prepared in an

* *Polymer-free formaldehyde specially prepared by the Standard Chemical Company, Montreal*

excess of methyl alcohol was compared with a commercial sample which had been exposed to low temperature in order to increase the amount of polymer. It was also of interest to ascertain the effect of variation in polymer content on the response to formaldehyde-hormone treatments.

The data on germination and dry weight of plants grown in soil (Table IV) are for two low commercial grades of wheat. They indicate that there is little difference in the response to these two different formaldehyde solutions. While the treatments in soil were not replicated, and consequently the data cannot be tested for significance, it is evident from the dry weights that somewhat greater damage to growth may be caused by the polymer-free formaldehyde. Improvement in germination and early growth from the use of formaldehyde-hormone solutions appears to be independent of the content of polymer.

TABLE IV

EFFECT OF COMMERCIAL AND POLYMER-FREE FORMALDEHYDE ON GERMINATION AND DRY WEIGHT OF COMMERCIAL WHEATS

Treatment	Hormone concentration, p.p.m.	Germination, %				Dry weight of plants from 50 seeds grown in soil, gm.	
		Germinator		Soil			
		No. 5 wheat, 1935	No. 6 Special wheat, 1935	No. 5 wheat, 1935	No. 6 Special wheat, 1935	No. 5 wheat, 1935	No. 6 Special wheat, 1935
Untreated control		84	84	90	94	0.72	0.52
Commercial formaldehyde control		67	68	86	82	0.52	0.43
Polymer-free formaldehyde control		72	65	82	82	0.40	0.38
Commercial formaldehyde and hormone*	0.01	76	82	86	92	0.71	0.46
	1.0	72	71	90	86	0.67	0.43
	3.0	78	79	88	80	0.66	0.48
Polymer-free formaldehyde and hormone*	0.01	74	91	88	90	0.65	0.46
	1.0	81	80	88	82	0.70	0.39
	3.0	82	86	88	88	0.57	0.53
Necessary difference, 5% level		8	8	—	—	—	—

* *No. 5 wheat treated with formaldehyde-indolylacetic acid; No. 6 special wheat treated with formaldehyde-naphthylacetic acid.*

Effects of Time Interval between Treatment and Seeding

It has been pointed out that formaldehyde treatment usually causes some injury to the seed, and that this is increased when seeding is delayed or the soil is dry. In consequence, the response of formaldehyde-hormone-treated seed to delayed planting, or planting in dry soil, is of interest.

Data are given in Table V for the germination of No. 1 Northern wheat treated by immersion. This experiment was one of the earlier ones. Others which it seems unnecessary to report showed that immersion and sprinkling

gave essentially similar results, and in later work the more convenient sprinkling method was used.

The data show that germination of the formaldehyde control is reduced when seed is planted one day after treatment, and that germination is further markedly reduced when the time between treatment and planting is extended to two and eight days. These effects are, however, substantially offset in all the formaldehyde-hormone treatments. A somewhat better response is obtained with naphthylacetic acid, particularly with seed planted eight days after treatment. However, individual blotting-paper flats of 100 seeds were used and the data cannot be tested for significance.

TABLE V

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON PERCENTAGE GERMINATION OF NO. 1 NORTHERN WHEAT PLANTED ONE, TWO AND EIGHT DAYS AFTER TREATMENT*

Treatment	Hormone concentration, p.p.m.	Germination, %		
		1 day	2 days	8 days
Untreated		82	—	—
Soaked in water only		74	74	66
Formaldehyde control		58	0	4
Formaldehyde and naphthylacetic acid	0.1	76	78	62
	1.0	82	86	66
	5.0	78	68	66
Formaldehyde and indolylacetic acid	0.01	68	78	52
	0.10	80	60	56
	1.0	72	58	50

* *Treatment by immersing the seed in all cases. Seed planted in germinator.*

Data are given in Table VI for the germination of Laurel (hull-less) oats on blotting paper and in wet and dry soil. The solutions used in these treatments were prepared from stock formaldehyde-hormone solutions which had been held in glass containers for 23 days. The germinator results showed improved germination for seed planted one and two days after treatment, and the improvement is somewhat more marked for the germination of seed planted in soil. Seed planted in dry soil was watered two days after planting. It is apparent that the two-day period in dry soil has not increased damage, but a longer period of exposure to this condition might have done so.

Improvement in germination over the formaldehyde control thus results from formaldehyde-hormone treatment even if planting is delayed for several days after treatment.

Effect of Storing Stock Solutions of Hormone Chemicals in Commercial Formaldehyde

It has been shown that freshly prepared formaldehyde-hormone solutions can be used to advantage in the treatment of seed. Since the practical

application of hormone chemicals for this purpose requires their addition to commercial formaldehyde by the manufacturer, and since several weeks usually intervene between packaging and use, stability of the chemical is of major importance. A series of experiments was carried out to determine the effect of storage period on the physiological activity of hormones dissolved in commercial formaldehyde.

TABLE VI

EFFECT OF FORMALDEHYDE AND FORMALDEHYDE-HORMONE TREATMENTS ON PERCENTAGE GERMINATION OF LAUREL (HULL-LESS) OATS PLANTED ONE AND TWO DAYS AFTER TREATMENT*

Treatment	Hormone concentration, p.p.m.	Germination, %					
		Germinator		Soil			
				Moist		Dry†	
		1 day	2 days	1 day	2 days	1 day	2 days
Untreated		96	90	92	98	88	98
Formaldehyde control		67	54	48	52	46	56
Formaldehyde and naphthylacetic acid	0.1	82	72	72	66	66	76
	1.0	78	84	88	80	72	84
Formaldehyde and indolylacetic acid	0.1	70	76	66	76	74	72
	1.0	78	84	54	68	84	76
Necessary difference, 5% level		9	–	–	–	–	–

* *The stock formaldehyde-hormone solutions had been held for 23 days in glass containers.*
† *The samples in dry soil were watered two days after planting.*

Hormone chemicals were added to formaldehyde solution containing 37% of the gas by weight, to give 3.2, 32 and 320 p.p.m. in the stock solutions. Subsequent dilution of 1 : 320 gave treating solutions containing 0.01, 0.1 and 1 p.p.m. of hormone.

The data in Table VII give mean root and stem measurements on 20 plants of wheat and oats selected at random from each lot, and compare the effect of seed treatment with fresh formaldehyde-hormone solutions and solutions from stock preparations held in glass containers for 10 weeks. Both wheat and oats show significant root damage from formaldehyde alone and this damage is significantly reduced throughout by the use of formaldehyde-hormone treatments. Improvement in oat stems is found with the new solutions, while the stem differences of wheat are not significant. It is evident that the physiological activity of naphthylacetic acid has been maintained for the period of 10 weeks, and that the differences between old and new solutions are not statistically significant. Since essentially similar results are obtained with stock solutions of indolylacetic acid, there is reason to think that the stability of the hormone chemical in commercial formaldehyde will be satisfactory for practical application in seed treatment.

Some indication of this was already afforded by the data in Table VI on the germination of hull-less oats treated with solutions made from stock preparations held in glass for 23 days. Since formaldehyde-hormone treatments with fresh solutions are not included, a direct comparison is impossible. However, the increase in germination from hormone treatment indicates that physiological activity is still in evidence after holding either indolylacetic or naphthylacetic acids in commercial formaldehyde for 23 days.

TABLE VII

EFFECT ON PHYSIOLOGICAL ACTIVITY OF STORING STOCK SOLUTIONS OF NAPHTHYLACETIC ACID IN COMMERCIAL FORMALDEHYDE FOR TEN WEEKS IN GLASS CONTAINERS

Solution	Hormone concentration, p.p.m.	No. 3 Northern wheat		Laurel (hull-less) oats	
		Root length, mm.	Stem length, mm.	Root length, mm.	Stem length, mm.
Untreated control		373	181	249	156
Formaldehyde control		291	164	154	145
Formaldehyde and naphthylacetic acid					
Stored 10 weeks	0.01	396	176	199	153
Fresh	0.01	350	164	229	161
Stored 10 weeks	1.0	370	174	231	156
Fresh	1.0	380	176	212	161
Necessary difference, 5% level		54	15	42	12

Effect of Hormone Application after Seed is Treated with Formaldehyde and Dried

It would seem that hormone chemicals in formaldehyde solutions either reduce initial seed injury or provide some essential factor which subsequently enables the plant partially to overcome existing damage. Stoichiometrical considerations make it unlikely that the hormone directly affects the activity of the formaldehyde itself. In the following experiments, two different samples of wheat were treated with hormones one day after formaldehyde treatment and drying. In the first experiment, treated seed was washed in water and a portion was washed in 0.01 p.p.m. naphthylacetic acid. The seed was planted seven days after this treatment, and the germination percentages were: formaldehyde control, 4%; washed in water, 44%; washed in 0.01 p.p.m. hormone solution, 56%.

In the second experiment, the seed was dusted with talc at ½ oz. per bushel, giving talc and talc-hormone treatments* of 10 and 50 p.p.m. of indolylacetic and phenylacetic acids. The seed was planted immediately and gave germination percentages of 38, 66 and 58 respectively. The resulting stimulation of early growth was as marked as the effect on germination. In consequence, it seems probable that the effect of hormone on formaldehyde-treated seed should be attributed to subsequent stimulation of damaged seed rather than to prevention of damage at the time of treatment.

* *Dust treatments refer to parts by weight of hormone chemical applied to a million parts of seed. Solution treatments, of necessity, merely indicate the hormone concentration.*

Experiments with Hormones in Seed Disinfection by Means of Copper Sulphate or Hot Water

Copper Sulphate Treatment

Data on germination and dry weight of plants from wheat treated with copper sulphate and copper sulphate-hormone mixtures are given in Table VIII. It is apparent that hormone has little effect on germination, though stimulation is suggested at three p.p.m. with No. 5 special wheat. An increase occurs in the dry weight of plants from 50 seeds, except with No. 5 special wheat at one p.p.m. of naphthylbutyric* acid.

TABLE VIII

EFFECT OF COPPER SULPHATE AND COPPER SULPHATE-HORMONE TREATMENTS ON GERMINATION AND DRY WEIGHT OF WHEAT PLANTED IN SOIL

Treatment	Hormone concentration, p.p.m.	Germination, %		Dry weight of plants from 50 seeds, gm.	
		No. 5 Special wheat, 1935	Feed wheat, 1935	No. 5 Special wheat, 1935	Feed wheat, 1935
Untreated control		92	92	0.56	0.64
Copper sulphate control		62	66	0.36	0.33
Copper sulphate and naphthylacetic acid	0.1	54	58	0.41	0.43
	1.0	62	58	0.40	0.43
	3.0	86	52	0.58	0.39
Copper sulphate and naphthylbutyric acid	1.0	52	52	0.33	0.47

Root and stem measurements are given in Table IX as means of 15 plants chosen at random from each treatment of feed wheat. Seed injury by copper sulphate is shown by significant reduction in the length of roots, and copper sulphate-hormone treatment increases roots significantly above the mean value for the copper sulphate control. The result at one p.p.m. is an exception, as the increase is not significant. It is evident that copper sulphate treatment has not reduced the stems of feed wheat, and with added hormone

TABLE IX

EFFECT OF COPPER SULPHATE AND COPPER SULPHATE-HORMONE TREATMENTS ON THE LENGTH OF ROOTS AND STEMS OF FEED WHEAT PLANTED IN SOIL

Treatment	Roots, mm.	Stems, mm.
Untreated control	306	207
Copper sulphate control	226	207
Copper sulphate and 0.1 p.p.m. naphthylacetic acid	275	231
Copper sulphate and 1.0 p.p.m. naphthylacetic acid	257	237
Copper sulphate and 3.0 p.p.m. naphthylacetic acid	272	219
Copper sulphate and 1.0 p.p.m. naphthylbutyric acid	305	240
Necessary difference, 5% level	41	18

* *The naphthylbutyric acid used in all these experiments is a mixture of 1- and 2-γ-naphthylbutyric acids.*

the stems of the treated samples are, with an exception at 3 p.p.m., all significantly longer than those of the untreated control.

Root and stem measurements also were made on 20 plants from the copper sulphate control and 3 p.p.m. naphthylacetic acid groups of No. 5 special wheat. The average roots were 226 and 314 mm. respectively, an actual difference of 88 with 55 mm. required for the 5% level of significance. Similarly, the stem increase of 27 mm. is significant.

Hot Water Treatment

In Table X are given the percentage germination in the germinator and in soil of three different wheats treated with hot water and hot solutions of naphthylacetic acid. The data for growth in soil show that the hormone treatment has increased the rate of germination with each sample of wheat, though there is no difference in the total germination of feed wheat. The germination on blotting paper is improved with feed and Huron wheats, but not with No. 5. While the blotting paper germination of No. 5 wheat fails to show improvement from hormone treatment, there was substantially better growth.

TABLE X

EFFECT OF HOT WATER AND HOT HORMONE SOLUTION TREATMENTS ON GERMINATION OF WHEAT

Variety or grade of wheat	Treatment	Germination, %					
		Germinator	Soil				
			Days after planting				
			5	6	7	8	20
Feed, 1935	Untreated control	72	72	84	86	88	88
	Hot water control	58	2	12	30	34	68
	Hot naphthylacetic acid solution, 1 p.p.m.	72	4	32	48	56	64
	Hot naphthylacetic acid solution, 10 p.p.m.	62	0	2	38	56	68
No. 5, 1935	Untreated control	73	66	86	86	86	86
	Hot water control	52	0	4	8	8	22
	Hot naphthylacetic acid solution, 1 p.p.m.	34	0	0	4	14	24
	Hot naphthylacetic acid solution, 10 p.p.m.	31	0	0	8	28	40
Huron	Untreated control	56	52	56	60	66	66
	Hot water control	10	0	4	4	4	22
	Hot naphthylacetic acid solution, 1 p.p.m.	36	4	16	28	34	40
	Hot naphthylacetic acid solution, 10 p.p.m.	36	28	50	58	58	64

Data are given in Table XI on root and stem measurements as means of 10 plants selected at random from each treatment. Hot water treatment of Huron and No. 5 wheat results in a significant reduction in the total length of roots and stems. Treatment in hot hormone solution increases the roots of No. 5 wheat significantly, but has little effect on the length of stem. There is a striking increase in the stems of Huron following hormone treatment. The increase in roots at one p.p.m. is not significant, while the 10 p.p.m. treatment gives roots significantly longer than those of the untreated control.

While the absence of damage to feed wheat by the hot water treatment cannot be explained, the lack of marked hormone stimulation is, as already indicated, the expected result in such circumstances.

TABLE XI

EFFECT OF TREATMENT WITH HOT WATER AND HOT HORMONE SOLUTION ON LENGTH OF ROOTS AND STEMS OF WHEAT PLANTED IN SOIL

Treatment	No. 5 wheat, 1935		Huron		Feed wheat, 1935	
	Roots, mm.	Stems, mm.	Roots, mm.	Stems, mm.	Roots, mm.	Stems, mm.
Untreated control	238	220	348	344	226	177
Hot water control	163	191	262	241	240	192
1 p.p.m. naphthylacetic acid	208	192	276	324	207	207
10 p.p.m. naphthylacetic acid	233	198	432	341	223	188
Necessary difference, 5% level	37	28	56	36	—	—

Determination of Physiological Activity by the Response of Formaldehyde-damaged Seed

The response of damaged seed may be used as a method of determining the physiological activity of chemicals (6). It is essential that seed susceptible to formaldehyde damage be used. The subsequent improvement effected by the test solution then gives a measure of its activity. The method is advantageous because of its simplicity and gives results which are in close agreement with those obtained by other methods.

A number of pure chemicals were tested for physiological activity by the response of low grades of wheat from the 1935 crop. Data on germination and dry weight of plants grown from 50 seeds in soil are given for a series of isolated experiments in Table XII. Duplicates of 50 seeds were germinated on blotting paper and the necessary difference for the 5% level of significance is given. Since the data for germination and dry weight of plants grown in soil are for individual flats, they cannot be examined statistically. Nevertheless, they serve to confirm the results obtained with the germinator. It will be observed that formaldehyde damage is significant throughout. With No. 5 special wheat (A), the greatest reduction in damage is effected by naphthylbutyric acid, phenylacetic acid (which has already been recognized as having activity) giving somewhat less protection against damage. Vanillin and methoxysalicylaldehyde are intermediate in effect, with benzoic acid and piperonal showing activity of a lower order. The improvement caused by coumarin, sulphanilamide (para-amino benzene sulphonamide), and Vitamin B_1 indicates physiological activity. Pyrrole acetic acid appeared to have no effect.

Data are given at the bottom of Table XII on the response to treatment with a mixture of one p.p.m. each of ethyl mercury bromide and naphthylacetic acid. While the use of organic mercurial dust disinfectants as carriers for

hormone chemicals has been reported (5), it is of some interest to determine the effect when formaldehyde solutions are fortified with a second disinfectant. The results with No. 4 wheat indicate that the addition of organic mercury does not restrict the activity of the hormone.

Data on germination, dry weights, and measurements on the roots and stems of wheat grown in soil are given in Table XIII. The germination and

TABLE XII

PHYSIOLOGICAL ACTIVITY OF VARIOUS CHEMICALS AS INDICATED BY THE RESPONSE OF FORMALDEHYDE-DAMAGED SEED

Variety or grade of wheat	Treatment*	Germination, %		Weight of plants, gm.
		Germinator	Soil	
No. 5 Special wheat (A), 1935	Untreated control	87	92	0.87
	Formaldehyde control	6	2	0.002
	Formaldehyde and naphthylbutyric acid	80	68	0.67
	Formaldehyde and phenylacetic acid	71	54	0.59
	Formaldehyde and piperonal acid	68	32	0.32
	Formaldehyde and benzoic acid	73	48	0.36
	Formaldehyde and methoxysalicylaldehyde	68	44	0.57
	Formaldehyde and vanillin	69	48	0.52
	Necessary difference, 5% level	9	—	—
No. 5 wheat, 1935	Untreated control	85	88	
	Formaldehyde control	0	0	
	Formaldehyde and coumarin	66	34	
	Formaldehyde and sulphanilamide	69	70	
	Necessary difference, 5% level	6	—	
No. 5 Special wheat (B), 1935	Untreated control	88	92	0.56
	Formaldehyde control	72	70	0.37
	Formaldehyde and colchicine	80	72	0.46
	Necessary difference, 5% level	5	—	—
Feed wheat, 1935	Untreated control	77	80	0.43
	Formaldehyde control	56	70	0.30
	Formaldehyde and Vitamin B_1	76	82	0.39
	Necessary difference, 5% level	10	—	—
No. 3 Northern wheat, 1935	Untreated control	90		
	Formaldehyde control	68		
	Formaldehyde and 0.1 p.p.m. indolylacetic acid	88		
	Formaldehyde and pyrroleacetic acid	66		
	Necessary difference, 5% level	11		
No. 4 Northern wheat, 1935	Untreated control	80	86	0.58
	Formaldehyde control	58	66	0.45
	Formaldehyde and ethyl mercury bromide and naphthylacetic acid	80	78	0.58
	Necessary difference, 5% level	8	—	—

* *1 p.p.m. of the chemical under test was added to the formaldehyde solution.*

TABLE XIII

EFFECT OF COLCHICINE AND VITAMIN B_1 ON GERMINATION, DRY WEIGHT, AND ROOT AND STEM MEASUREMENTS OF FORMALDEHYDE-DAMAGED WHEAT PLANTED IN SOIL

Variety or grade of wheat	Treatment	Germination, %	Dry weight of plants from 50 seeds, gm.	Root length, mm.	Stem length, mm.
Feed, 1935	Untreated control	92	0.64	306	207
	Formaldehyde control	66	0.36	196	204
	Formaldehyde containing 1 p.p.m. colchicine	86	0.58	266	223
	Necessary difference, 5% level	—	—	41	18
Huron	Untreated control	82	1.04	399	243
	Formaldehyde control	54	0.46	261	216
	Formaldehyde containing 1 p.p.m. Vitamin B_1	72	0.63	317	236
	Necessary difference, 5% level	—	—	63	22

dry weight data are essentially similar to those of Table XII. There is a statistically significant decrease in roots from formaldehyde treatment of both feed and Huron wheat. While colchicine effects a significant increase in both roots and stems of feed wheat, the improvement effected by Vitamin B_1 on Huron wheat is just below the level of significance.

Effect of Hormone Solutions on Untreated Seed

A number of control experiments were made in order to test the effect of hormone solutions on untreated seed. The data are summarized in Table XIV, which gives percentage germination and dry weights, and some green weights, of plants grown from three commercial grades of wheat treated with solutions of hormone. There is no significant difference in germination of No. 5 wheat, but an increase at 0.01 and 3 p.p.m. with No. 6 special. The dry weights of plants of these two samples, grown in soil, fail to suggest appreciable increase from hormone treatment. It is apparent that germination and green weights of No. 1 Northern wheat show a significant amount of damage from hormone treatment, the damage reaching a peak at 50 p.p.m. These observations indicate that the response of damaged seed to treatment with solutions of hormones must be associated with the damaged condition, rather than to any normal growth stimulation.

It is also interesting to note that when seed is treated with hormone or formaldehyde-hormone solutions and dried previous to germination, a reduced growth of common molds is frequently observed. This phenomenon has not yet been investigated quantitatively. However, as the effect of these solutions on the growth of molds is a matter of some importance, the results of our preliminary qualitative investigation are reported in order to illustrate the sterilizing effect of naphthylacetic acid.

Duplicate malt agar plates of (A) untreated wheat, (B) wheat surface sterilized by soaking for 15 minutes in calcium hypochlorite solution (2%

TABLE XIV

EFFECT OF SOLUTIONS OF HORMONE ALONE ON GERMINATION AND WEIGHT OF WHEAT

Treatment	Hormone concentration, p.p.m.	Germination, %					Weight of plants from 50 seeds grown in soil		
		Germinator		Soil			Dry weight, gm.		Green weight, gm.
		No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 1 Northern wheat*	No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 5 wheat, 1935†	No. 6 Special wheat, 1935*	No. 1 Northern wheat*
Untreated control		84	84	98	90	94	0.72	0.52	12.6
Hormone solutions	0.01	85	93		96	94	0.77	0.54	
	1.0	76	88		96	92	0.71	0.51	
	3.0	88	93		96	96	0.67	0.46	
	5.0			91					12.5
	10.0			92					12.4
	20.0			89					12.3
	50.0			77					9.6
	100.0	80		90	90		0.69		11.6
Necessary difference, 5% level		8	8	7					1.3

* *No. 1 Northern wheat and No. 6 Special wheat treated with naphthylacetic acid.*

† *No. 5 wheat treated with indolylacetic acid.*

available chlorine), and (C) wheat soaked 12 hr. at 32° C. in a 100 p.p.m. solution of naphthylacetic acid, are shown in Plate I. It is apparent that the hormone treatment reduces the growth of molds without reducing the development of bacterial colonies. Although general conclusions cannot be drawn from the results of this and other similar experiments made so far, the evidence suggests that further investigation might be profitable.

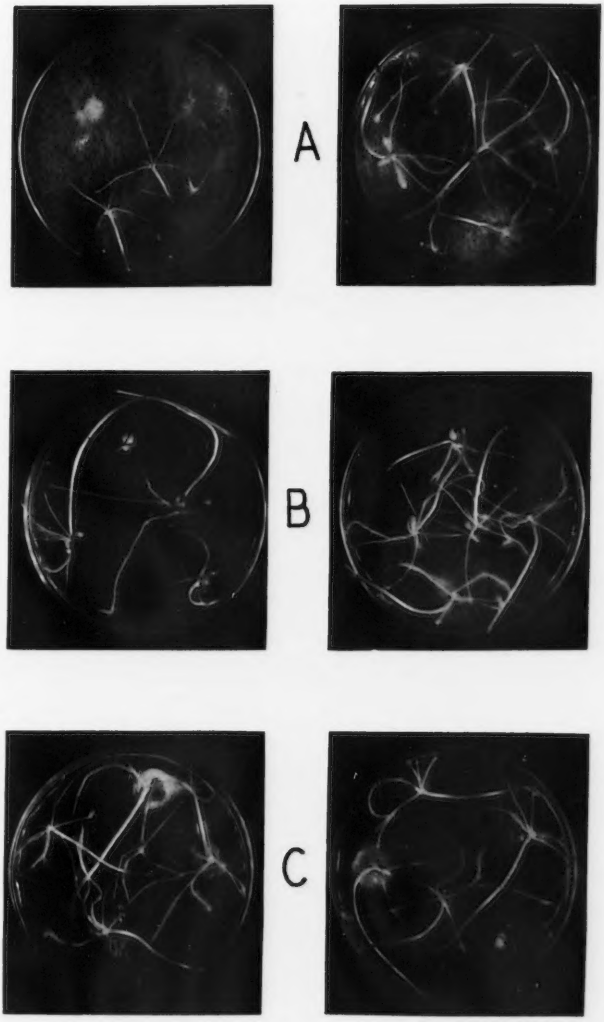
General Discussion

Seed injury from disinfection with formaldehyde causes reduced root growth, and generally, some reduction in the length of stem of 2- to 3-weeks-old wheat and oat plants. Increased root development is the most marked response after addition of physiologically active chemicals to the disinfectant solution. There is also a tendency to increase the stem length. These observations would appear to substantiate Henry's suggestion that formaldehyde damage is due to the inactivation of the seed's natural growth hormone. The large number of substances capable of reducing the injury, and the absence of inactivation when indolylacetic acid is added in the formaldehyde solution, suggest that the response is not so simple. It is possible that a precursor, or accessory factor, of the normal growth-promoting substance is affected by the formaldehyde. The mechanism may involve the enzyme system.

A recent paper by Clark (4) suggests that functional materials such as hormones, vitamins, bios, auxins and growth regulators manifest themselves through the activation of some enzyme. Clark's suggested mechanism for the action of these substances fits in with the observations of Atwood (1), who found that the diastatic activity of formaldehyde-treated grain is retarded. Whether injury is a direct effect on the auxins, or is related to some accessory factor, or to changes in the enzyme system, the addition of hormone chemicals permits readjustment and thereby reduces damage. It is apparent that the application of physiologically active chemicals to reduction of seed injury should be helpful in elucidating the mechanism involved.

The practical application of hormones in seed disinfection offers interesting possibilities. While laboratory observations have been limited to the effect on germination and early growth, field experiments must determine the effect on incidence of smut and final yield. It is evident that much of the objection to the formaldehyde treatment has been removed if results in the field are essentially similar to those in the laboratory. It will be possible for the manufacturer to add the required amount of chemical to commercial formaldehyde at the time of final packing, since the chemicals can be held in this way for at least 10 weeks without loss in activity. This procedure should eliminate the hazard of damage from overdosage and render the use of hormones in seed treatment exceedingly simple.

There are similar possibilities in the use of hormone chemicals in the hot water treatment for loose smut. As this method must be used when seed is infected with smut, and serious seed injury may result, the use of hormones should increase the margin of safety as well as improve the growth from the seed which does germinate.



Effect of hormone solution on mold growth on Huron wheat seedlings grown six days on malt agar gel. A. No treatment. B. Surface-sterilized with calcium hypochlorite solution. C. Treated with 100 p.p.m. naphthylacetic acid solution.

Acknowledgments

The writer wishes to express his appreciation to Dr. J. A. Anderson for assistance in preparing the manuscript and to Mr. A. Sirianni for his work in the laboratory.

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A METHOD OF DETERMINING THE MEAN SPEED OF MOVEMENT OF INSECTS IN A MASS OF FLOUR[1]

BY JOHN STANLEY[2] AND B. N. SMALLMAN[3]

Abstract

A method of determining the mean speed of movement of adults of the flour beetle *Tribolium confusum* Duv., moving through a mass of flour, is described. The method consists of allowing a beetle to wander for a suitable length of time through a mass of flour made up of alternate black and white layers of flour, each 1 mm. thick. The motion of the beetle's legs churns the two colours into one another as it tunnels through the flour. If the flour mass be compressed into a solid cake, and sectioned at right angles to the laminae, the course of the tunnel is marked by gray traces owing to this mixing. From a study of these traces in section, the trail may be reconstructed and the distance traversed in unit time computed.

Introduction

In the course of certain investigations of the growth of populations of the flour beetle, *Tribolium confusum* Duv. (Stanley, 1–3), one of us (J.S.) required a value for the mean speed of movement of the adult beetles as they wandered at random through the flour. Following numerous unsuccessful attempts, the writers have developed a very satisfactory method based on an idea conceived by the senior author. Briefly, the method consists of laying down a laminated mass of flour consisting of alternate black and white layers, each 1 mm. thick. A beetle is allowed to bore through this mass for a suitable time, and during this journey, the motion of the beetle's legs churns the two colors into one another along the path, so that if the mass is later compressed into a hard block and sectioned at right angles to the laminae, traces of the trail can be seen as gray marks on the faces of the sections. From a study of these traces the trail can be reconstructed, and the distance travelled in unit time computed.

Experimental Methods

The laminated flour mass is laid down in a heavy-walled steel mold (Fig. 1) made from a short length of seamless steel tubing. The removable bottom is held in place by six heavy machine screws with their heads countersunk into the under side of the bottom. The dimensions of the mold are: inside

[1] *Manuscript received June 3, 1938.*

Contribution from the Department of Biology, Queen's University, Kingston, Ontario, Canada.

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diameter, 3 in.*; outside diameter, 4.5 in.; wall thickness, .75 in.; inside depth, 4 in.; thickness of bottom, .75 in.

When the flour mass is to be compressed, a solid steel piston (Fig. 1) is inserted in the top of the mold. The dimensions of the piston are: length, 4.75 in.; diameter for 4 in. of the length, 2.95 in., for the remainder, 3.25 in. A pressure of 100,000 lbs. is applied in a large press used at Queen's University for testing the strength of materials. As the total area of the top of the mass is 7.0686 sq. in., a pressure of approximately 14,128 lb. per sq. in. is exerted. This produces a hard chalk-like cake which is expressed from the mold, after removal of the bottom, by use of an arbor press. Both piston and mold are very accurately machined and are heavily nickel-plated over an undercoat of copper.

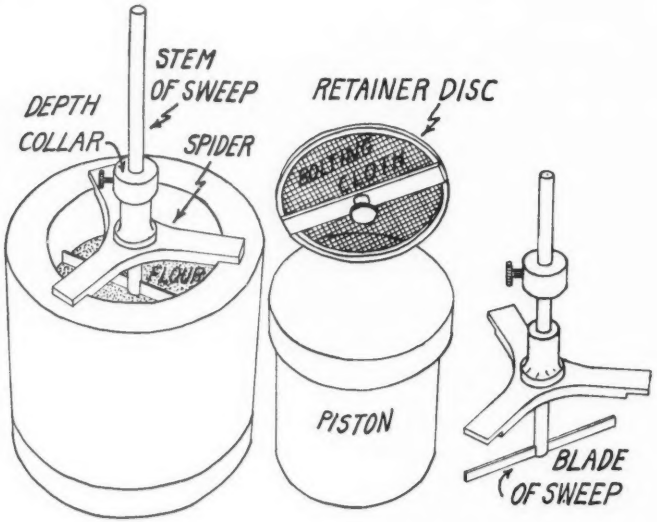

FIG. 1. *Complete assembly of mold, spider and sweep; retainer disc; piston; sweep assembly.*

The laminae are laid down as follows: a brass spider (Fig. 1) fits accurately into the top of the mold and holds a "sweep" with its stem (.25 in. diameter) accurately in the centre line of the mold. The sweep can be supported with the bottom edge of its blade at any height above the bottom of the mold by setting a small collar at the appropriate graduation mark on the stem. The 1-mm. graduations of the stem are not marked in Fig. 1 as they are too fine to show properly. Having set the sweep blade just 1 mm. above the bottom of the mold, a measured amount of white flour sufficient to make a layer

**The mold was constructed with a diameter measurable in inches because it was at first intended to use laminae .05 in. thick. The change to metric measurements was made because of the ease of obtaining high grade 1-mm. co-ordinate paper.*

1 mm. thick over the bottom is poured in and is then smoothed off by cautiously rotating the sweep to and fro. Some trouble has been experienced through tearing of the first lamina, owing to the small coefficient of friction between the flour and the smooth bottom of the mold. However, when the first lamina has been laid down the others follow easily, with successive 1-mm. elevations of the sweep blade for each layer. Small quantities of extra flour are removed with a small shovel shaped like a hoe. The black layers are made from flour into which 1.5% of purified lamp-black has been intimately mixed in a pebble mill. Altogether 91 layers are laid down, Nos. 1, 3, 5, ... 91 being white, Nos. 2, 4, 6, ... 90 being black.

The beetle is then placed on top of this flour mass and watched until it bores in, whereupon a retainer disc (Fig. 1), consisting of a brass ring covered with silk bolting cloth, is gently lowered onto the flour to prevent the beetle from coming out and wandering on top without boring.

After a suitable period, usually 100 hr., during which the assembly is held at constant temperature, compression is carried out as described above, following which the resultant cake is glued into a hardwood cradle to support it during the subsequent sectioning.

Section at right angles to the laminae is effected by routing off successive 1-mm. layers by means of a routing cutter rotating at 5,000 r.p.m. in a Delta Triple Duty drill press. It is interesting to note that the mass is very abrasive, and rapidly dulls the cutter. The senior author, who is now carrying on the work, is considering the use of a cutter tipped with stellite.

As each face is exposed, it is mapped on 1-mm. co-ordinate paper, and these maps are subsequently trans-illuminated in pairs and larger groups to see that the traces line up properly, and that no small parts have been missed. Occasionally a few millimetres are missed. These parts are inked in by hand, but as they are small and of obvious size and shape this does not result in any appreciable loss of accuracy. Unfortunately the sections cannot be reproduced by photography, because the downward movement of the upper laminae during compression produces a central "dishing" of the laminae in the middle of the block. In section therefore, the upper and lower laminae appear as straight lines, being in contact with parts of the mold during compression, while the middle layers are curved. It is necessary for purposes of computation to transpose the maps of the sections to co-ordinate paper having straight lines for all laminae, homologous with the original flat form of the laminae before compression.

These retouched maps are then traced with india ink on to 1-mm. sheets of "lumarith", a transparent celluloid-like material. The necessary 76 sheets of lumarith are previously cut about 3.1 in. by 93 mm., stacked up, and clamped together. The edges of the resultant block are carefully machined and polished to form a rectangular block 3 in. by 3 in. by 91 mm., *i.e.*, just the size to enclose the uncompressed flour mass within rectangular planes, with the component layers of lumarith at right angles to the black and white flour laminae. When the traces are marked on these prepared sheets,

and the sheets stacked, a transparent block results with a black replica of the beetle's trail suspended within its substance.

Two procedures are subsequently followed. If simply the length of the trail produced per unit time is desired, the co-ordinates of numerous points along its centre-line are found from a study of the traces in the dissectible lumarith block, and the sum of the distances between them computed from the familiar formula of co-ordinate geometry. From this the distance travelled in unit time is readily found.

If a solid model of the trail is desired, the traces are cut through each sheet by means of a small router, the sheets restacked, and melted Wood's metal is injected into the resultant worm-like cavity. The lumarith is then cut away, and the step-like annulations running around the model smoothed off with a high-speed dental burr. Owing to the waste of expensive lumarith, it is not planned to make many such models.

Owing to the extraordinarily laborious nature of the process, only one block has as yet been run through, but others are being worked on. When sufficient data has accumulated to compute a statistically sound value for the mean speed, this value will be reported.

Acknowledgment

The writers are indebted to the National Research Council and to the Science Research Committee of Queen's University for financial support in this and related work with *Tribolium*. They are also indebted to Miss Isobel Hope and Miss Margaret Biehn for their patient and meticulous labor in building up the laminations, and to Professor D. Ellis of Queen's University for the use of the press. The mold and accessory equipment were made by Mr. Bradfield, the Research Mechanic at Queen's University.

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AN ACCOUNT OF A PARASITIC COPEPOD, SALMINCOLA SALVELINI SP. NOV., INFECTING THE SPECKLED TROUT[1]

By Laurence R. Richardson[2]

Abstract

An account and description are given of *Salmincola salvelini* sp. nov., a parasitic copepod (Lernaeopodidae) infesting the speckled trout (*Salvelinus fontinalis* Mitchill) at Gaspé, P.Q., and also taken from arctic charr (*Salvelinus alpinus*) collected at Sugluk Bay, northern Labrador. *S. salvelini* is found in the mouth of both hosts where it is firmly attached to the tissues of the roof and sides of the mouth and tongue. It does not occur on the gills, and in this respect is distinct from *Salmincola edwardsii* commonly found on speckled trout in North America. *S. salvelini* has a marked superficial similarity to *S. gibber*, from which it can be distinguished by the presence of a spine on the terminal segment and a papilla on the penultimate segment of the maxillipeds.

Introduction

Records of parasitic copepoda infesting the speckled trout (*Salvelinus fontinalis* Mitchill) to the present time appear to be restricted to the two species, *Salmincola edwardsii* (Olsson) (Lernaeopodidae) and *Argulus canadensis* Wilson (Argulidae). The material described in the present paper constitutes a third and apparently undescribed species for which the name *Salmincola salvelini* is proposed.

Argulus canadensis Wilson has been reported from speckled trout at Cape Breton, where it infests many of the fresh-water fishes (Wilson, 6). *S. edwardsii* is a common and frequently serious parasite of the speckled trout and has a wide distribution on this continent (Davis, 1). In the Province of Quebec, *S. edwardsii* (recognised by fishermen as the bug, or "bebitte") is occasionally found on trout living under natural conditions; and in certain lakes (Spider Lake, Frontenac County, and adjacent small lakes to the south, as well as several lakes in the vicinity of Murray Bay, Charlevoix County) *S. edwardsii* produces or contributes to a significant mortality amongst the adult trout.

Salmincola salvelini, on the other hand, has been collected from speckled trout in the Provincial Fish Hatchery at Gaspé, and from arctic charr (*Salvelinus alpinus*) taken at Sugluk Bay, northern Labrador. The former specimens were sent to the author by Mr. R. C. Lindsay, Superintendent of the Provincial Fish Hatchery at Gaspé. The latter specimens were found in charr belonging to the collection of the Institute of Parasitology, Macdonald College. The specimens from both districts show similar variation in form and possess similar mouth parts.

In both cases the parasites were restricted to the mouth, being attached to the membrane lining the mouth and hanging with the body free in the buccal cavity, anterior to the insertion of the gills. Many specimens were

[1] *Manuscript received March 17, 1938.*
Contribution from the Department of Zoology, McGill University, Montreal, Canada.

[2] *Assistant, Department of Zoology, McGill University, Montreal, Canada.*

attached to the tongue and alongside this structure; a smaller number occurred on the roof and sides of the buccal cavity.

S. salvelini shows a marked superficial dimorphism. From the one host it is common to obtain specimens with an orbicular, depressed trunk and others with a narrower, rectangular and deeper trunk. This dimorphism is apparently correlated with the position of attachment of the parasite in the mouth of the host, the flattened orbicular forms being attached to the tongue, while the rectangular forms are generally found attached to the sides or roof of the mouth. Several specimens, taken from the groove at the side of the tongue where they were subjected to mechanical pressure during closure of the mouth of the host, possess a normal cephalothorax and a markedly distorted trunk.

The orbicular forms have a superficial resemblance to *Salmincola gibber*, although the cephalothorax is not as wide in proportion to the width of the trunk as in this latter species. The mouth parts of the two species are also distinct. The first antenna of *S. salvelini* bears distally three spines which lack the segmentation and fringe of hairs shown for the corresponding structure of *S. gibber* (Wilson, 2). The second antennae of the two species are quite distinct from one another. In *S. gibber*, the dorsal ramus of this appendage is in the form of a large flattened claw bearing two spines on the concave margin. The same structure in *S. salvelini* is segmented, and consists of a broad basal segment bearing a squat papilla and a small spine, and a terminal segment in the form of a strong claw-like spine. The ventral ramus in *S. salvelini* is unsegmented and bears only a single simple spine, while the ventral ramus in *S. gibber* is segmented, having a large basal segment and a smaller terminal segment which is fringed with fine hairs. In addition, there is a small palp situated on the ventral aspect of the proximal portion of the appendage. This is not described for *S. gibber*.

The mandibles of both species are similar, although in *S. salvelini* the ventral edge of the shaft is straight, not concave as in the other species. The maxillae of *S. salvelini* lack the protuberance seen on this structure in *S. gibber* and are also distinct in having two of the distal spines mounted on inflated bases. The arms of *S. salvelini* are relatively narrower than in *S. gibber* and the dorsum of the cephalothorax can be clearly seen in lateral view.

The most readily observed distinction between the two species is found in the maxillipeds. The maxillipeds of *S. salvelini* bear on the terminal segment a small spine, and on the penultimate segment a broadly conical papilla. These structures are quite obvious without dissection and have no counterparts in *S. gibber*.

Wilson (5) has listed further a total of eight species of *Salmincola* from arctic waters, where it is apparent that the genus is well represented. The short and broad cephalothorax, the simple form of the trunk and the mushroom-like bulla of *S. salvelini* sets this species apart from other arctic forms with the exception of *S. gibber*, from which, as shown above, it is clearly

distinct on the basis of the mouthparts as well as minor relative proportions in form.

The present collection contains 22 specimens, five of which were taken from arctic charr collected at Sugluk Bay. The remainder were collected at Gaspé. Some of the material fixed in formalin is badly shrivelled and the measurements have been made from other material preserved in alcohol.

Description

Genus *Salmincola* Wilson 1915.

Fixed parasitic copepods with the cephalothorax lacking a carapace and separated from the unsegmented, stout trunk by a groove or neck, with the maxillipeds inside of the second maxillae, the latter being longer than the cephalothorax and situated close behind the mouth tube.

Salmincola salvelini SP. NOV. Fig. 1, A–H.

The male has not yet been collected.

The females are of moderate size, the cephalothorax and trunk measuring 6.0 mm. in an average specimen. The cephalothorax (Fig. 1, B) is almost as wide as long and has slightly concave lateral margins. Between the proximal ends of the second maxillae the cephalothorax is swollen markedly, giving much the appearance of heavy shoulders when seen in lateral view. The cephalothorax is joined to the trunk by a short and broad "neck". The trunk is variable in shape; on the one hand many specimens have a trunk markedly flattened and circular in dorsal view, while many others have a more elongate, narrower trunk, rectangular when seen in dorsal view. In the latter specimens the trunk is not markedly depressed. The trunk lacks any posterior process. The egg-sacs are long and stout, generally approximating (in orbicular forms) the combined length of the cephalothorax and trunk, or exceeding it (in rectangular forms). The trunk and egg-sacs in specimens attached to the tongue are frequently deformed.

The first antennae (Fig. 1, D) are small, unsegmented, and tipped with three small spines. The first antennae are the only appendages, with the exception of the second maxillae, that are visible in a dorsal view of the cephalothorax. The second antennae (Fig. 1, E) are sturdy and laterally compressed. Segmentation is obscure. The ventral ramus of the second antenna is a simple conical extension ending bluntly with a smooth surface and bearing distally a single weak spine. The dorsal ramus is a two-jointed conical structure, the proximal segment being inflated and bearing ventrally a low conical papilla and a short spine. The distal segment forms a strong, conical, slightly asymmetrical cone. Close to the base of the dorsal ramus and on the lateral aspect, the proximal portion of the appendage bears a low, rounded protuberance covered with short spinules which form a hispid patch. Proximal to this hispid protuberance and also on the lateral aspect there is a small palp-like structure bearing five or more small spines. The terminal spines, and papilla of the dorsal and ventral rami are curved slightly so that their tips point away from the mouth tube.

The mandibles (Fig. 1, G) are short, broadest at a point half-way from their base, and beyond this taper sharply to the small rounded distal end. The ventral margin is straight. The teeth are few and recurved, increasing in size from the small distal tooth to the third, which is the largest. The two most proximal teeth are weak.

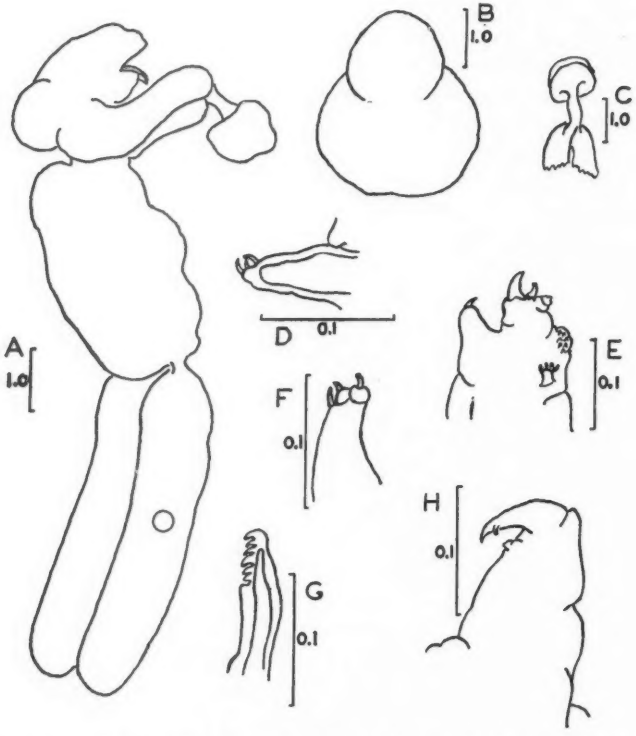

FIG. 1. *A. Lateral view adult female Salmincola salvelini. B. Cephalothorax in dorsal view. C. Distal ends of second maxillae showing bulla. D. First antenna seen from the lateral aspect. E. Second antenna from lateral aspect, showing the proximally situated palp-like structure. F. The first maxilla from the lateral aspect. G. Mandible. H. The maxilliped from the ventral (posterior) aspect.*
N.B. *The scales are in millimetres.*

The maxillae (Fig. 1, F) are small and short, bearing distally three small spines, two of which are mounted on bulbous expanded bases. The second maxillae vary from strongly curved, short "arms" approximately half the length of the trunk (orbicular forms), to long, slender, and almost straight "arms" subequal to the length of the trunk (rectangular forms). The bases of the second maxillae are not markedly swollen. The bulla is of moderate size, petioled and mushroom-shaped. (Fig. 1, C.)

The maxillipeds (Fig. 1, H) are stout. The penultimate segment bears on its lateral surface a broadly conical papilla tipped with a very fine spinule or hair. It is but little longer than the terminal segment, which has a concave margin and bears a strong spine on the medial surface.

The cephalothorax averages 2.5 mm. long by 2.35 mm. wide. The trunk is 4.5 mm. long by 3.0 mm. wide and 2.5 mm. deep in forms with a rectangular trunk, and 3.5 mm. long by 3.5 mm. wide and 1.5 mm. deep in forms with a circular trunk. The egg-sacs, in forms with a rectangular trunk, are 8.0 mm. long and 1.5 mm. wide, while in forms with a circular trunk the egg-sacs are slightly wider but only 5.0 mm. long. Eggs in the egg-sac are 0.3 mm. in diameter.

In all available material *S. salvelini* has been found attached to the tongue and tissues lining the buccal cavity back to the beginning of the pharynx only. The author is indebted to Mr. Lindsay for the statement that even in heavily infected trout, *S. salvelini* is not present on the gills. In this habit *S. salvelini* differs markedly from *S. edwardsii*, from which it may be distinguished on sight by the smaller size and different habit of the latter.

Wilson (4), in giving a list of new hosts for parasitic copepods, records the collection of *S. gibber* from the mouth and tongue of the charr *(Salvelinus alpinus alipes)* taken at Cairn Lake and Konochickalak Lake by the MacMillan Baffin-land expedition.

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THE NORTHERN FOWL MITE (*LIPONYSSUS SYLVIARUM* C. & F., 1877)

INVESTIGATIONS AT MACDONALD COLLEGE, QUE., WITH A SUMMARY OF PREVIOUS WORK[1]

By Donald Cameron[2]

Abstract

This paper gives a complete review of past work on the northern fowl mite. The generic name for the species is *Liponyssus* Kolenati, 1859. To show the geographical distribution of this mite, 20 bird and two mammalian hosts are given in systematic order. A seeming discrepancy in previous descriptions of the protonymph is figured and described. A description of the larva and of the males, resembling those of *L. bursa*, is given with figures. They were taken, with males of *L. sylviarum*, from fowls at Macdonald College, but all females taken from the birds are as described for *L. sylviarum*. Average duration of the egg stage is 30.4 hr. and of the larval stage 8.33 hr. at 100°–104° F. and 90–100% relative humidity. All attempts at artificial feeding failed. This mite does not aestivate and will not breed upon chicks. It multiplies rapidly, spreads readily from bird to bird, and survives long periods of starvation, but temperatures below 7° F. cause death in a short time, when away from the host. The high thermal death point lies between 104.2 and 108.5° F. The economic importance is uncertain but might be great. Control is cheaply and easily accomplished by the use of nicotine sulphate.

Introduction

Since 1920, when Wood first collected the northern fowl mite (*Liponyssus sylviarum* Canestrini and Fanzago, 1877) from domestic poultry in the United States, much has been written about it but much work still remained to be done. In 1922, Caesar reported it from poultry at Guelph, Ontario, and since then it has appeared in other parts of Canada, where it has been reported as doing serious damage to the domestic hen.

The Quebec Department of Agriculture provided the funds for the following investigation, which were made at Macdonald College, Que., between October, 1935 and May, 1937.

In this paper the author has tried to review all past work and from this review and his own observations to clarify the synonymy and give as complete a host list as possible; distinguish between this mite and the tropical fowl mite (*Liponyssus bursa* Berlese, 1888); describe the morphology, life history and habits; determine the economic significance; and state the best means of control. A complete bibliography is also given.

Synonymy

1877 *Dermanyssus silviarum;* Canestrini and Fanzago. Atti ist. Veneto sci. 5 : 4 : 124.

1884 *Leiognathus silviarum;* Canestrini. Atti ist. Veneto sci. 6 : 2 : 1573-1660.

[1] *Manuscript received February 15, 1938.*
Contribution from the Department of Entomology, Faculty of Agriculture of McGill University, Macdonald College, Que. Macdonald College Journal Series No. 94.
[2] *Assistant in Zoology, Cornell University, Ithaca, New York.*

1885 *Leiognathus silviarum;* Canestrini, Prospetto dell' Acarofauna Italiana, 121.
1889 *Leiognathus sylviarum;* Berlese, Acari Myriopoda et Scorpiones hucusque in Italia reperta. 53 : 5 : 19.
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1922 *Leiognathus sylviarum;* Ewing, Proc. U.S. Nat. Mus. 62 : 13 : 7.
1926 *Liponyssus sylviarum;* Vitzthum, Seckenb. Frank.-a-M. 8 : 30-39.
1931 *Liponissus sylviarum;* Vitzthum, Z. Parasitenk. 4 : 1 : 9-11.

The genus *Liponissus* was erected by Kolenati, in 1858, from a male specimen of *Dermanissus setosus* Kolenati, 1856. In 1859 he gave three drawings of the monotype, including a dorsal view of an adult and a ventral view of a nymph or (more probably) of a female. He also altered the spelling, at this time and thrice later, to *Liponyssus*, which spelling has since been retained (incorrectly according to Vitzthum (55)) in the literature. In a letter to the writer, Dr. C. W. Stiles states that he had adopted Liponyssus on the basis that Kolenati's papers are inconsistent, that the earliest *nyssus* in this group is *Dermanyssus*, and that (since Kolenati in 1858 uses *nyssus* in six generic names and *nissus* in only one) the derivation of *nissus* is identical with that of *nyssus*. Thus *nissus* is clearly an error of transcription and therefore subject to correction.

It is no longer possible to identify the type species *Liponyssus setosus* Kolenati, 1859. Kolenati's only record is from a Siberian horse-shoe bat, *Rhinolophus clivosus*, and he handed over no co-types to the large museums. Besides, according to Vitzthum (55), his description does not agree with his figures and the latter are insufficient.

In 1877, a written description of a new species was published, and this species was named *Dermanyssus silviarum* by Canestrini and Fanzago.

Canestrini in 1884 formed a new genus *Leiognathus* for *Liponyssus arcuatus* Koch, *L. silviarum* C. & F., and *L. uncinatus* sp. n. He repeated the written description of *L. silviarum* in another publication (1885), and Berlese (3) redescribed this mite as *Leiognathus sylviarum* and added figures of dorsal and ventral sides of the female, and of the epistome, peritreme and chelicera. He repeated this description in 1893.

Vitzthum (53) redescribed the female with figures of ventral and dorsal aspects and the ventral plate, besides describing the protonymph for the first time, with figures of the dorsal and ventral aspects, peritreme and tarsus. He included *Leiognathus* Canestrini, 1884, in *Liponyssus* Kolenati, 1859, and so the species became *Liponyssus sylviarum* (Canestrini and Fanzago, 1877).

As sole difference between *Leiognathus* and *Liponyssus*, Ewing (19) states that in the females of *Leiognathus* "the body is constricted suddenly and is provided with an incomplete transverse groove behind the insertion of the

[1] *The mite described by Megnin in 1891, under the name Lophoptes patavinus, as causing a special acariasis in Paduan fowls, is possibly L. sylviarum (Hirst, 26).*

last pair of legs." According to Vitzthum (54), this cannot be verified in *Leiognathus sylviarum* unless the female is fully mature and gravid, and even then it is only suggested.

Vitzthum (54) states that the horn-shaped apophysis of the palpus-trochanter of the female of *Liponyssus sylviarum* (also a character of the genus *Ceratonyssus*), should it occur again in females of species having undivided dorsal shields, must cause further separation of the genera in question.

Geographical Distribution with Hosts in Systematic Order

A. True Hosts

Aves		
Gallinae (fowl-like birds)		
Phasianidae (poultry)		
Gallus domesticus L. (domestic fowl)		
U.S.A.—		
Md., Beltsville Ill., Raymond	—coll. Wood (59) det. Hirst (24) (first as a variety of *L. bursa*, later as *L. sylviarum*).	On fowls and inside straws in nests.
Ind., Lafayette	—Troop (51)	On fowls only.
N.Y., (northern) Minn., Minnesota	—Bishopp (5)	On fowls and in nests.
Ind., Bloomfield Ill., Harvel N.Y., Plattsburg Ohio, Oxford	—Cleveland (15) (uncertain whether dealing with *L. sylviarum* or *L. bursa*.	On fowls and in nests.
N.Y., Closter N.Y., Ithaca	—Matheson (35)	?
Va. N.C. Fla.	—Kaupp (30)	On fowls and in nests.
Ohio, Wooster	—Cutwright (16, 17) (uncertain, calls mite the "feather mite", sometimes "the tropical fowl mite" *L. sylviarum*)	On fowls and in nests.
Kansas, Manhattan	—Payne (42) (calls it *L. sylviarum* but Bushnell and Brandly (9) call it *L. bursa*).	On fowls, in nests, on dropping boards, in cracks in roosts and walls.
Mass., Agr. College	—Payne (43) (calls it *L. sylviarum* of Cleveland (15) but later was uncertain whether he had *L. bursa* or *L. sylviarum*)	On fowls, in nests, on dropping boards, and in cracks in roosts and walls.
Canada—		
Ont., Guelph Ont., Port Dover	—Caesar (10)	On fowls and in nests.
B.C., New Westminster	—Spencer (48)	On fowls and in nests.
Que., Macdonald College	—Maw (37)	On fowls and in nests.
England—		
Bedfordshire, Bletsoe	—Hirst (27).	?
Dorsetshire	—Taylor (50)	On fowls only.
Columbae (pigeons and doves)		
Columbidae (pigeons)		
Europe England	—Hirst (26)	On birds only.

Aves—*Continued*		
Pici (woodpeckers)		
Picidae (woodpeckers)		
Dryobates villosus villosus L. (hairy woodpecker)		
Canada—		
Que., Macdonald College	—Rayner (44)	On bird.
Colaptes auratus auratus L. (flicker)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird.
Macrochires (swifts, goatsuckers, etc.)		
Micropodidae (swifts)		
Chaetura pelagica L. (chimney swift)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird.
Passares (perching birds)		
Tyrannidae (tyrant flycatchers)		
Tyrannus tyrannus L. (kingbird)		
Canada—		
Que., Macdonald College	—Rayner (44)	On bird.
Sturnidae (starlings)		
Sturnus vulgaris L. (European starling)		
U.S.A.—		
—	—Matheson (35)	On bird.
Ohio, Wooster	—Cutwright (as above for *G. domesticus*).	On bird.
Canada—		
Que., Macdonald College	—Rayner (44)	On bird.
Icteridae ("troupials")		
Molothrus ater ater Bodd. (cowbird)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird.
Euphagus carolinus Müller (rusty grackle or blackbird)		
Canada—		
Que., Macdonald College	—Rayner	On bird.
Quisculus quiscula quiscula L. (purple grackle)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird and in nest (heavy).
Fringillidae (finches)		
Passer domesticus L. (European sparrow)		
U.S.A.—		
Md., Beltsville Ill., Raymond	—coll. Wood (59) det. Hirst (24)	On birds (light), nestlings and nests (heavy).
—	—Bishopp (5)	On nestling.
Canada—		
Que., Macdonald College	—Rayner (44)	On bird and in nest.
Hirundinidae (swallows)		
Progne subis subis L. (purple martin)		
Canada—		
Que., Macdonald College	—Rayner (44)	On bird.
Hirundo erythrogastra Bodd. (barn swallow)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird.
Mniotiltidae (wood warblers)		
Dendroica aestiva aestiva (Gmelin) Baird (yellow warbler)		
Canada—		
Que., Macdonald College	—Maw *et al* (38)	On bird.
Motacillidae (wagtails and pipits)		
Motacilla alba L. (European white wagtail)		
Russia—	—Hirst (24)	In nest.
Mimidae (mocking birds)		
Dumetella carolinensis L. (catbird)		

Aves—*Concluded*		
Passares (perching birds)—*Concluded*		
Canada—		
Que., Macdonald College	—Rayner (44)	On bird and in nest.
Sylviidae (kinglets and gnatcatchers)		
Sylvia atricapilla L. (blackcap warbler)		
Italy—		
Pisa	—Canestrini and Fanzago (12)	On bird and in nest.
Sylvia curruca L. (lesser whitethroat)		
Germany—		
Weimar	—Vitzthum (53)	In an old nest.
Turdidae (thrushes and their allies)		
Planesticus migratorius migratorius L. (robin)		
Canada—		
Que., Macdonald College	—Rayner (44)	On bird and in nest.
Turdus merula L. (blackbird)		
Italy—		
Portici	—Leonardi (33)	?

B. Accidental Hosts (Biting but no Apparent Multiplication on Host)

Mammalia		
Rodentia		
Muridae		
Dicrostomyx hudsonicus (Labrador collared lemming) det. Anderson.		
Canada—		
Que., Macdonald College	—coll. W. E. Whitehead, Nov. 19, 1935. Det. H. E. Ewing, 1933 (unpublished).	
Primates		
Hominiidae		
Homo sapiens (man)		
U.S.A.—		
—	—Riley and Johannsen (45, p. 265).	Bites caused pruritis (intense itch).
Ill., Raymond	—Wood (59)	Bites.

Wood (59), at Raymond, Ill., reports negative records for the brown thrush, song sparrow, blackbird, redheaded woodpecker, screech-owl, quail, robin, mouse nest, and mole.

Bishopp (5) states that *L. sylviarum* has never been recorded in southern latitudes. However, the first record came from Pisa, Italy, in 1877, and Kaupp (30) cites records from Maryland, Illinois, Indiana, Virginia, California and Florida.

Payne (42) gives negative records for the English sparrow at Manhattan, Kansas, and at the Massachusetts Agricultural College (43). He states that climate seems to be no barrier to the distribution of *L. sylviarum*. However, it is uncertain whether he was working with *L. bursa*, *L. sylviarum*, or both; and Bushnell and Brandly (9), also at Manhattan, Kansas, declare that the mite reported by Payne as *L. sylvarium* was probably *L. bursa*.

The tropical fowl mite *Liponyssus bursa* was first reported in 1888 from poultry in Buenos Aires, Argentina, South America. Since then Hirst (22) reports it on poultry in South America, Africa, Mauritius, China and India. Records are also given of specimens from starlings, sparrows, the "hibon" (Comoro Islands), from a lizard and from man (India and Zanzibar).

Hirst (23) and Roberts (46) report it from poultry in Australia, and the latter also reports it from pigeons and sparrows. Definite instances of attacks upon man are reported from China by Hirst (25), from Australia by Cilento (13) and Roberts (46). The latter states that *L. bursa* attacks any animal in the house, but only lives ten days away from the host and cannot breed in the meantime.

During the winter months, wild birds found around the poultry plant at Macdonald College are limited to starlings and sparrows. *L. sylviarum* has been recorded from both these hosts (Rayner (44)), but the records were made during the summer months. The writer examined sparrows and starlings during the winter months: 16 sparrows yielded nothing, one of 13 starlings carried a very light infestation of *L. sylviarum*, and 30 pigeons gave negative records, as did 7 rats.

Differences between *L. sylviarum* and *L. bursa*

L. bursa has three pairs of hairs on the sternal plate; two pairs of long hairs at the posterior tip of the dorsal shield; and (in the male) no transverse line posteriorly, on the ventral plate, in front of the anal plate. *L. sylviarum*, on the other hand, has but two pairs of hairs on the sternal plate; one pair at the posterior tip of the dorsal shield; and (in the male) has a transverse line present, posteriorly, on the ventral plate, in front of the anal plate.

Hirst (26), although he points out the above differences, states that perhaps *L. bursa* is a variety of *L. sylviarum*. The significance of this should not be overlooked, since *L. bursa* is suspected of disease transmission from rodents to man, and *L. sylviarum* is here reported from a Labrador collared lemming.

Among mites collected from fowls at Macdonald College, the writer found all the females to agree with the above-mentioned figures and descriptions of *L. sylviarum*; but while some of the males were as figured for *L. sylviarum* by Hirst (26), others were more like those which he figured as being *L. bursa* (*cf.*, Fig. 1B in this paper, with Fig. 69 by Hirst (26)).

V. Morphology, Life History, and Habits

A. Morphology

General

A translation from the Italian of the original written description of *L. sylviarum*, Canestrini and Fanzago (12), is as follows:

"Body oval, elongate; slightly restricted behind the shoulders, and rounded and slightly notched at the posterior margin. A small seta on the shoulder, on each side, and another small one in front of this. The whole margin of the abdomen bears moderately stout setae, which increase in robustness from front to rear, the last pair being situated on the posterior margin, above the anus and extending posteriorly. These two setae are distinguishable from the others by their pronounced extra length. The abdominal surface is smooth. The legs are all covered with uniform setae except in the fourth pair, which, in the penultimate segment, are consistently longer and more curved."

"The colour, of our example, bleached to a uniform white in alcohol.

"Length: one mm.

"Habitat: found on *Sylvia atricapilla* (collector: Professor Richardi)."

In 1884, Canestrini gave a written description of the male and female, with five drawings to illustrate the latter sex.

Berlese (3, 4) gives a written description of the female with figures. He states that Canestrini (11) probably studied a replete nymph and not a female.

Vitzthum (53) gives descriptions and figures of ventral and dorsal views of female and protonymph, and criticizes the description of the female by Berlese. He states that Berlese failed to notice certain hairs on the dorsal shield and on the soft-skinned dorsal area, that the peritreme was wrongly described, and that, on the ventral side, only the anterior half of the genital plate was noted. Vitzthum also says that it is doubtful if Canestrini had seen the male, and that the protonymph can no longer be known as the female.

The egg, larval, protonymphal, deutonymphal and adult stages are mentioned by Wood (59). His material was identified by Hirst (25), who showed the morphological differences between *L. bursa*, *L. sylviarum* and *L. bacoti* (26).

In 1923, Cleveland figured and described the egg, larval, protonymphal, deutonymphal and adult stages of a mite which he states is either *L. bursa* or *L. sylviarum*, but his figures are scarcely adequate.

Vitzthum (55) states that "Von keiner *Liponissus*—Art kennt man eine Deutonympha. Dieses Stadium scheint übersprungen zu werden", and "Eine Protonympha dagegen besitzen alle Liponissiden und wahrscheinlich ist dies das Stadium, in dem sie zur Welt kommen. Eier legen sie keinesfalls, und eine frei lebende Larva ist noch nicht gefunden worden."

Maw, Whitehead and Bemont (38) give a photo of egg masses on feathers, and state "in colour the adults are darkish brown which shows some variations, with a distinct colour pattern which also varies apparently with age".

Egg

Vitzthum's statements of 1931 contradict those made by previous workers. For photographs of egg masses see Wood (59) and Maw, Whitehead and Bemont (38). Cleveland (15) gives a figure of the egg of either *L. bursa* or *L. sylviarum*.

The writer has observed oviposition by *L. sylviarum* females kept in isolation in small vials, and larvae have been seen in the act of hatching from the eggs.

Larva

An accurate figure is given here (Fig. 1A) of the dorsal aspect of a larva of *L. sylviarum* prior to moulting to the nymphal stage. This larva is colorless, six-legged, and has no plates on either ventral or dorsal surfaces. At the posterior end of the body are three pairs of stout bristles of equal length, and smaller bristles cover the rest of the body and legs. The chelicerae are non-functional, and the first pair of legs seems to be considerably longer than those of the nymphal stage. The developing chelicerae, palps and four pairs of legs of the nymphal stage are shown in this figure, and the fourth pair has just begun to straighten out and break through the larval skin.

Protonymph

The pair of long bristles, which Vitzthum (53) shows as arising from the posterior end of the protonymph, are found by the writer to arise from the posterior dorsal shield. The latter (Fig. 1C) is also different in shape to that figured by Vitzthum.

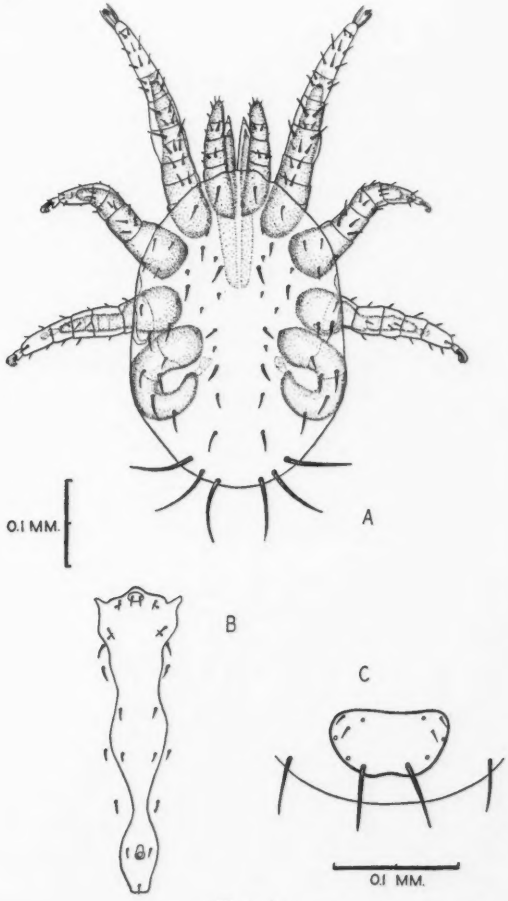

FIG. 1.

Deutonymph

Apparently no one has accurately figured or described this stage, and since the writer can find no form which he can positively identify as the deutonymph, he does not feel prepared to say at present whether there is such a form.

Adult

See Vitzthum (53) for accurate figures and a description of the female.

Hirst (26) has figured and described the differences between both sexes of *L. sylviarum* and *L. bursa*. As already mentioned, the author has taken, from fowls at Macdonald College, some males resembling those figured by Hirst as *L. sylviarum* and others which are more like his figures of *L. bursa*.

Fig. 1B shows the ventral plate of a male mite, and is more like Hirst's figure for *L. bursa* than that for *L. sylviarum*. In shape it is less angular than *L. bursa*, but the margin is more sinuous; just in front of the anus the plate is narrower than that of *L. sylviarum*, and there is no indistinct suture present.

In his figure of the male of *L. sylviarum*, Hirst (26), although he does not remark upon it in his description, shows eight pairs of hairs on the ventral plate anterior to the anus, while in *L. bursa* the eighth pair are shown at the sides of, and not on, the ventral plate. In Fig. 1B of this paper it will be seen that the second, third, sixth and eighth pairs of hairs arise off the ventral plate.

All females examined were those of *L. sylviarum*.

B. Life History

Egg

Oviposition occurs on the fowl or on the inside of straws in the nest, and the eggs adhere by means of a sticky substance, according to Wood (59). Troop (51) and Taylor (50) found them on the fowl only, and Caesar (10) states that they are found at the base of the feathers. Hatching occurs in three days off the host, according to Wood (59). Cleveland* (15) found that 36 to 96 hr. were required, while Cutwright* (17) agrees with Wood.

In the case of *L. bursa*, the eggs are laid away from the host, according to Hirst (22).

In January, 1936, the writer made preliminary experiments which indicated that the duration of the egg stage was from 30 to 31 hr. Accurate experiments were then made as described below.

Oviposition occurred in a dark incubator at a temperature of 100–104° F. Several females were placed in a tube plugged at the mouth by a wad of wet cotton wool wrapped in wet black silk. A similar wad was placed at the foot of the tube so that the relative humidity, inside the tube, was 90 to 100%. Examinations were made very 20 min. by means of electric light and a hand lens. Each egg was transferred by a camel's hair brush to a separate tube similar to the one in which it was deposited, where it was observed during hatching. The larval stage was observed until the time of moulting.

No female was observed to lay more than one egg in captivity; and since females could not be fed and recovered, the number of eggs laid per female was not determined. The mites congregated at certain points in the tube and laid their eggs in clumps. Deposition of an egg was almost instantaneous, but each female remained over her egg, or, if disturbed, made a short "tour

* *In this and subsequent references marked with an asterisk, there is doubt whether L. bursa or L. sylviarum was described by the author.*

of inspection" and returned to the egg which she "fingered" alternately with left and right fore-legs.

When hatching, the egg splits in the horizontal plane across the posterior end and along both sides for over half its length. This splitting is brought about by the larva, which pushes backwards against the posterior end of the egg and then backs out of the shell. The average duration of the incubation period was 30.4 hr. (see Table I).

TABLE I

DURATION OF EGG AND LARVAL STADIA

Time of oviposition	Duration of egg stage, hr.	Duration of larval stage, hr.	Total time, hr.	Remarks
13/1/36				
1. 5:30 p.m.	27.75	8.75	36.75	
2. 6:00 p.m.	27.25	Died	—	
14/1/36				
3. 12:30 p.m.	29.5	9	38.5	Egg deposited in drop of water.
4. 12:30 p.m.	32.25	7.75	40	Struggle to hatch.
5. 1:30 p.m.	30.25	8.25	38.5	Egg deposited in drop of water.
6. 1:30 p.m.	34	7.75	41.75	Hatching took 15 min.
7. 1:30 p.m.	33.75	8	41.75	Egg deposited in drop of water.
8. 2:30 p.m.	28.75	Died	—	Egg deposited in drop of water.
9. 6:30 p.m.	30	7.25	37.25	
10. 6:30 p.m.	30.5	10	40.5	
Average	(10) 30.4	(8) 8.33	(8) 39.34	

Larva

The larva does not feed, and moults in 17 hr., according to Wood (59). Cleveland* (15) states that the larva moults in less than a day. Preliminary experiments, made by the writer, gave the larval stage as 9 hr.; more accurate experiments, described above, indicated that the average duration of the larval stage is 8.33 hr. (Table I).

The larva may remain quiescent beside the egg or (presumably to find more favorable conditions) may move to a different location. Its movements are sluggish, and activity seldom occurs for long. At the posterior end of the larva, two bud-like structures appear and grow into the fourth pair of legs, which break through the larval skin and enable the protonymph to emerge backwards through this slit.

Protonymph

Since the protonymphs could not be induced to feed artificially, and since they could not be recovered as identified specimens when placed on a clean bird, the writer has failed to work out the life history from this point onward. According to Wood (59), the moult occurs in one to two days, or (Cleveland (15)) in two to three days.

Attempts to feed the protonymphs artificially were made as follows:

1. Defibrinated hen's blood was placed in a tube over the mouth of which a membrane was stretched. The tube was inserted through a cork which was fitted into one end of a glass cylinder containing starved mites. The other end of the cylinder was covered with black cloth. The apparatus was placed in the dark in an incubator at the temperature of the bird's body (100–104° F.). The membranes used were fresh sparrow skin, fresh starling skin, fresh chicken skin, fine parchment, fine rubber membrane, and collodion membrane. The mites would not feed.

2. A small area near the tail head of a live bird was cleared of feathers and ringed round with vaseline within which starved mites were placed. They refused to feed, some even crossing the vaseline to reach the shelter of the feathers.

3. A small area beneath the wing of a live bird was cleared of feathers, and a rubber suction cap containing starved mites was attached. Even after several hours, the mites did not feed.

Deutonymph

Moulting occurs in three to four days (Cleveland* (15)). Vitzthum (55) states that "Von Keiner *Liponissus*—Art Kennt man eine Deutonympha."

For reasons already stated, the writer is not prepared to say whether there is a deutonymphal stage.

Adult

There is no information on the duration of this stage.

C. Habits

General

Usually, moulting occurs on the host (Wood (59), Troop (51), Taylor (50)). Feeding occurs both day and night (Wood (59), Cleveland* (15), Maw (38)), the mites being found on the body of the fowl at all times (Troop (51), Bishopp (5), Cutwright* (16), Taylor (50)). Mites are found in the nest as well (Caesar (10), Bishopp (5), Cutwright*(16)), and as many as 50 mites on a new-laid egg is a characteristic sign of infestation (Cutwright* (16)). Payne* (42) and Maw (38) state that mites are found in the nests and on the dropping boards, roosts, and in the cracks in the walls of poultry houses. According to Cleveland* (15), they do not breed in such places, and Wood (59) states that although live mites may be found on loose feathers in the shade, none are found alive on grass or in sunny places. Some of the mites, on a loose feather placed on the back of a bird standing in the sun, died before they could crawl beneath the hen's plumage.

Caesar (10) noted that mites come to the surface of the bird's plumage and bask in the sun, and Maw (36, 37) states that they are often seen on the surface of a hen's plumage, especially if the bird is brought into a warm atmosphere.

On the fowl, mites seem to prefer the vent region (Wood (59), Caesar (10), Hirst (27), Maw (38)), and accumulate on a few feathers rather than on

many (Wood (59), Hirst (27)), although in heavy infestation they spread to other parts of the body (Wood (59)).

Mites feed intermittently, in patches the size of "a quarter", according to Wood (59).

Seldom are mites found on young chicks—probably due to lack of downy feathers—and an attempt to infest such chicks failed (Wood (59)). An attempt to infest 12-week-old White Leghorn cockerels failed, although mites were placed in the birds' feathers and on the roosting boards and floor of the house (Maw (38)). Payne* (42), on the contrary, found mites on all ages and both sexes of chickens and states that they thrive on capons.

This parasite multiplies very rapidly (Hirst (27)), the entire life cycle being 8 to 12 days (Cleveland* (15)).

After exposure in a bottle to a temperature of 7° F., or after 18 days in a bottle at laboratory temperature, mites survived (Caesar (10)). Cleveland* (15) and Maw (38) remark that mites live a long time away from the host, and Payne* (43) noted that the survival period was from two to three weeks at laboratory temperature. Twinn (52) reported that infestation by *L. sylviarum* of a church at Bell's Corners, Ontario, occurred after nestlings had flown from the numerous nests around the eaves. This infestation disappeared two or three weeks later.

In the late fall, after poultry is in winter quarters, mites appear. Occasionally they leave a bird almost as suddenly as they attack it (Payne* (42)), and some hens may be heavily infested while others seem to be free of parasites (Wood (59), Payne* (43)). Apparently fowls that dust themselves most are freest (Wood (59)). Some mites readily leave an infested fowl if the latter is handled (Wood (59), Taylor (50), Maw (37)) and may bite through tender human skin (Wood (59)). Twinn (52) states that numbers of *L. sylviarum* crawling over his hands did not bite.

As compared with the above-mentioned habits of *L. sylviarum*, Hirst (22) states that *L. bursa* infests the nest and surroundings of fowls and only attacks the fowls when food is required. When the birds leave the nest, the mites attack any animal or person in the house, but live only 10 days off the host and cannot breed in the meantime.

Maw *et al.* (38) believe that *L. sylviarum* aestivates during the summer months. This belief is apparently based on the fact that mites were not noticed upon birds during the summer months (although no specific search seems to have been made on the birds). During the summer, however, *L. sylviarum* was found on wild birds and in their nests.

From verbal reports it appears that *L. sylviarum* first appeared at Macdonald College in the fall of 1930, on seven White Leghorn male birds which had just arrived from the Central Experimental Farm, Ottawa. These birds were washed in a sulphur bath, but the infestation persisted; after spending the winter (1930–31) in a colony house, the birds were distributed over the college poultry plant and were used for breeding purposes. Since this time, sporadic outbreaks of mites have occurred.

Aestivation

Investigations were made to discover if *L. sylviarum* aestivates. During the fall of 1935, poultry houses which had been infested the previous spring were thoroughly examined. Material was taken from under and on floors, from between the walls, from cracks in roosts and from the roof, subjected to heat in a Berlese funnel, and chilled in an incubator. No signs of *L. sylviarum* were observed.

After birds had been placed in their winter quarters, an infestation of *L. sylviarum* was found on pullets. No mites were found anywhere except on the birds. Of 51 birds, 15 were infested (one extremely heavily and the others to varying degrees). By January 11, 1936, all but one were infested to a greater or lesser degree.

During the summer months of 1935 these pullets had been in contact with other birds in the college orchards and possibly with wild birds. Probably one or more of the pullets picked up mites from these sources, and the mites spread to the other birds during the winter.

The following experiments were made to determine if aestivation occurs:

Experiment 1. Nine male birds were used. All birds happened to be infested with body lice *(Eomenacanthus stramineus)*, which were killed by treating with sodium fluoride, the birds then being cleansed of the insecticide.

The birds were kept in individual coops under similar conditions in an empty pen. Three were placed on one side of the pen and used as controls, six were placed on the opposite side and infested with *L. sylviarum* (on March 7, 1936) by placing infested feathers in their coops and brushing the birds with them. This was repeated until all six birds were infested to a greater or lesser degree.

One bird died on April 2. The remaining five were still infested on June 15. At this time mites were found crawling all over the pen, and some attacked and infested two control birds, the third having died the previous day. On July 18, another of the controls died. The remaining six birds were now all heavily infested. One died on July 22, and one of the remainder was placed outdoors until October 1, (the cage being covered during cold or wet weather). On August 15, another bird died. Its tracheae were blocked with mucus, and the mite infestation was very heavy. Another bird died on October 21. It and the three live birds were still infested, but the bird which had remained outside from July 22 to October 1 was now carrying very few mites and showed no signs of infestation on November 9.

The other two birds remained infested until treated with nicotine sulphate on December 10 and 21 respectively, when the experiment was terminated.

Experiment 2. On March 11, 1936, a heavily-infested bird was placed in a vacant fumigation chamber at a temperature of 93° F. A dish of water was supplied. Twenty-four hours later many mites were discovered on the water, while many more were found in folds in the paper beneath the bird. The paper was removed to an incubator at 78° F. and relative humidity of 50 to 70%. It was stored for about seven months and examined. The mites were shrivelled and would not revive at lower temperatures.

Experiment 3. All stages of mites were left in an incubator at 60–65° F., with damp debris and a dish of water, from May 4 to October 6, 1936. The mites were then apparently dead, and did not revive after cooling to 30° F.

Experiment 4. On April 10, 1936, a White Leghorn pullet was placed in a wire cage, underneath which was a double bottom of beaverboard. Holes were punched in the upper layer, and some cotton wool was placed between the two bottoms. The whole was set on four small blocks in a shallow pan, which was kept full of water throughout the experiment. The environmental temperature was about 40° F. Mites could not escape from this cage, since tests have shown that they will not cross water. The pullet was heavily infested with mites the next day, and was kept in this cage until it died during the first week in June, with many live mites still actively feeding and breeding upon it. The cage was kept with all debris in it and surrounded by water until October 6. The false bottom was then examined thoroughly, but all mites were dead and none recovered at temperatures of about 105° F.

From the above results one may conclude that *L. sylviarum* does not aestivate, and it is possible that birds, when outside during the summer, get rid of most of their mites due to increased health and greater cleanliness; *e.g.*, when taking dust baths a bird may shake off many of its mites, and leave them to perish in the hot sun.

Examinations of birds under natural conditions at Macdonald College revealed that some carried heavy infestations of *L. sylviarum* during June, 1937. In the first week of October, 1937, 10% of a flock of 10,000 White Leghorns at Hartford Mills near Cornell University, Ithaca, N.Y., were found to be heavily infested with this mite by Dr. Robert Matheson, who very kindly took the writer with him on a visit to this flock. The seasons at which these natural infestations occur are further indications that the mite does not aestivate but remains upon certain birds throughout the summer.

Infestation of Baby Chicks

On March 19, four chicks (19 days old) were placed in a vacant fumigation chamber at 100° F. with a gentle current of air flowing through it. A dish of water and some "Lakko" chick feed were supplied. Several attempts were made to infest these chicks by brushing them with infested feathers, by covering the floor with infested feathers, and by placing the chicks for short periods in a paper bag containing infested feathers, at 71.6° F., but mites could not be induced to breed upon the chicks, although some fed upon them. The chicks were left in these infested surroundings. Two weak chicks died on the fourth day, but the other two lived and thrived until removed six weeks later, when they showed no traces of mites.

Progress of Infestation

On Dec. 21, 1936, one of 19 Barred Plymouth Rock pullets, No. 932, was found to be carrying a very heavy infestation of mites, while the others were apparently free. In three pens of Barred Plymouth Rock females, all but six birds carried a very heavy infestation of lice, as did many of the White Leghorn females in two pens. The rapidity of spread and fluctuation of

TABLE II

RAPIDITY OF SPREAD AND FLUCTUATION OF INFESTATION

No. of bird	1936		1937					
	Dec. 21	Dec. 24	Jan. 3	Jan. 7	Jan. 16	Jan. 22	Jan. 29	Feb. 4
932	v.v. heavy	v.v. heavy	v.v. heavy	v.v. heavy	v.v. heavy	light	light	light
945	–	light	f. heavy	f. heavy	f. heavy	v. heavy	v. heavy	heavy
946	–	light	v. heavy	v. heavy	v. heavy	v.v.heavy	v.v. heavy	light
929	–	light	light	f. heavy	f. heavy	f. heavy	f. heavy	f. heavy
943	–	light	light	f. heavy	f. heavy	heavy	v.v. heavy	f. heavy
942	–	light	light	light	light	light	f. heavy	f. heavy
935	–	light	light	light	light	v. light	v. light	v. light
936	–	light	light	light	light	light	f. heavy	f. heavy
931	–	light	light	light	light	light	v.v. light	v. light
941	–	light	light	light	light	light	f. heavy	f. heavy
948	–	–	v. light	v. light	v. light	v. light	f. heavy	f. heavy
942	–	–	v.v. light	v.v. light	v.v. light	v.v. light	f. heavy	f. heavy
950	–	–	v. light	v. light	v. light	v. light	light	light
937	–	–	–	v. light	v. light	v. light	v. light	light
938	–	–	–	–	light	v. light	v. light	v.v. light
940	–	–	–	–	–	v.v. light	v.v. light	v. light
933	–	–	–	–	–	–	f. heavy	f. heavy
934	–	–	–	–	–	–	light	light
949	–	–	–	–	–	–	v.v. light	clean
944	–	–	–	–	–	–	v.v. light	clean

v = *very*; f = *fairly*.

infestation in this house was recorded. The results are given in Table II. These show that infestation spreads rapidly from bird to bird, and that the degree of infestation increases rapidly on some birds, remains fairly constant on others, while certain birds keep clean.

Length of Survival Period Away from Host

Experiments were made to determine how long *L. sylviarum* will survive away from its host. Mites were subjected to the low temperatures of an empty laying house, the fairly constant temperature of the laboratory. and a series of constant high temperatures.

A. Low Temperatures

(i) Preliminary experiments. Mites in a glass tube were exposed for one hour at 8° F.; all died even when subsequently held at 70° F. On the other hand, exposure at 14° F. for one hour seemed to do no harm, since all mites recovered normal activity after 30 to 60 min. at 70° F.

(ii) On Dec. 29, 1936, mites were placed in small glass cylinders, both ends of which were closed by black cloth. The cylinders were divided into three groups; Group I was placed amongst the roof straw of a poultry house, Group II in holes punched in the Insulex material of the walls, and Group III in the floor debris, which had remained undisturbed since the fowls left it in spring. Each group consisted of the following: mites at all stages, egg masses, larvae, protonymphs, unfed adults, and replete adults. There were two cylinders of each of these, and feathers were added to one of each pair.

A hygro-thermograph was placed on the floor beside Group III and was covered lightly with floor debris. A maximum-minimum thermometer was suspended on the wall between Groups I (roof) and II (wall).

Mites on infested birds in the same house served as controls.

After 24 hr. at temperatures ranging between 16 and 28° F. and relative humidities of 64 to 84%, most of the mites in all stages recovered following 2 to 3 hr. in the laboratory at 70° F. and relative humidity of 20 to 50%. Eggs, however, were not observed to hatch. A few controls had also died.

Eggs and dead mites of all stages were replaced by fresh specimens in all groups and the cylinders were replaced. During the next 153.5 hours, the temperature varied from 10 to 46° F. with relative humidity of 54 to 100%. The mites were then brought into the laboratory at 70° F. and relative humidity of 20 to 50%, and left there under observation for two days. No mites of any stage recovered in Groups I and II, but in Group III, which had been in more humid surroundings, a few adults recovered normal activity. The tubes were placed for 18 hr. at 105.8° F. and relative humidity of 90 to 100%. There were still no signs of recovery after 10 days in Groups I and II, but a few more adults recovered in Group III. No eggs or immature stages had recovered. Controls were still alive at this time.

(iii) Cylinders of mites were divided into three groups. In each group, ten cylinders each contained about 100 active mites and many eggs, while an equal number contained, in addition, roof debris (Group I), wall debris (Group II), or floor debris (Group III).

Groups I, II and III were placed in the roof straw, in holes in the walls, and in the floor debris, respectively. Mites on infested birds were used as controls.

A hygro-thermograph and maximum-minimum thermometer were used as in (i).

The experiment lasted for 10 days (Jan 30–Feb. 9, 1937). The temperature fluctuated from 8 to 36° F. and the relative humidity from 50 to 90%. The cylinders were then placed at 96–104° F. and 90–100% relative humidity for about 24 hr., during which time they were kept under observation. About two-thirds of the adults in Group III recovered, most of which were in the cylinders without debris. No other mites survived and no eggs hatched. Mites were still present on the birds.

(iv) Ten cylinders of mites on feathers were placed in the floor debris of the above poultry house on Feb. 22, 1937.

Temperature and humidity records were kept as above.

Three weeks later, all mites were dead. The floor temperature, during this period, had fluctuated between 7° and 48° F. and the relative humidity between 40 and 77%.

Controls were still alive and normal.

B. Laboratory Temperature

All stages of mites were kept with feathers in bottles at 70° F. (bottles opened momentarily from time to time, to keep air fresh). Of the mites in

five bottles kept 19 to 30 days, none survived. In one bottle kept 11 days, 25% of the fed adults survived.

All stages of mites, kept on the surface of water in a dish, were dead in 11 days at 70° F.

Controls on the fowls under the same conditions were still alive.

C. High Temperatures.

All stages of mites, in each of 3 bottles, were dead in ½ hr. at 122–126° F.

All stages died in 1 hr. in a bottle at 116–122° F.

All stages died in 3½ hr. in a bottle at 111.2° F.

All stages died in 5 hr. in a bottle at 110.3° F.

All stages died in 3 hr. in a bottle at 108.5–110.3° F.

All stages died in 5 hr. in a bottle at 108.5° F.

All stages survived at 104.2° F., and therefore the high thermal death point of this species lies between 104.2° F. and 108.5° F.

VI. Economic Significance

A. Poultry

Historical

Infestation of poultry by a mite definitely identified as *L. sylviarum* is confined to the domestic hen (*Gallus domesticus* L.). All workers state that it causes economic loss to a greater or lesser degree (*cf.* previous literature). Heavy infestation may cause the fowl to die (Wood (59)), or to become so weak that it is unable to stand (Taylor (50)). Maw (37) asserts that vitality is reduced through loss of blood. Besides, egg production is reduced (Hirst (26, 27)), and these mites cause bloody scabs on the skin of the bird (Hirst (27)) owing to secondary infection of the bites (Whitehead (56)). Scabs appear chiefly on the back, wing joints and tail head (Maw (38), whose paper contains photographs showing scabs on plucked fowls; Payne* (42)). Cleveland* (15) observed bloody scabs on the skin of the bird.

Some birds are more heavily infested than others, according to Wood (59). Wood thinks that fowls which dust themselves most are freest, and he and Maw (38) state that cocks seem to carry a relatively heavier infestation than hens. Payne* (43) states that while some fowls may be heavily infested others seem to be free of parasites.

Wood (59) found few mites of *L. sylviarum* on young chicks, and Bishopp (5) states that none are found on baby chicks. On the other hand, Cleveland*(15) says that young birds are infested, and Payne* (43) asserts that the death loss in young chicks is very heavy, and all ages of males, females and capons are attacked.

Transmission of spirochaetosis from bird to bird by *L. bursa* was suggested by Hirst (22) and Roberts (46). In 1936, Brody proved that "fowl-pox" may be transmitted from bird to bird if *L. sylviarum* mites, fed on an infected bird, are crushed and inoculated into a healthy bird 4 days after the infective meal; but adult mites, fed on a healthy bird 4 and 11 days after their last association with a diseased bird, did not cause pox.

Post Mortems

Post mortems were performed on the following mite-infested birds during 1936.

(1) Feb. 19, White Plymouth Rock pullet.

Liver—slight lesions of *Histomonas meleagridis*.

Caecum—light infestation of *Heterakis gallinae*.

Duodenum—inflamed 3 in. from gizzard, owing to infestation with *Capillaria* spp. (diarrhoea symptoms evident before death).

Oviducts—highly fibrous-diseased.

Other organs—normal.

L. sylviarum infestation heavy (duration unknown). Death was not due to mites, but might have been caused by the *Capillaria* spp. or the diseased condition of the oviducts, or both.

(2) Feb. 22, White Plymouth Rock pullet.

Duodenum—very haemorrhagic (diarrhoea symptoms evident before death)—probably *Capillaria* spp., as many birds in the same pen showed diarrhoea symptoms and eggs of *Capillaria* spp. were found in the faeces of some.

Infestation with *L. sylviarum* very light; death probably not due to mites.

(3) Feb. 22, White Plymouth Rock pullet.

Bird too fat.

Heart slightly enlarged.

Trachea very haemorrhagic and blocked with bloody exudate.

Caecum—a few *Heterakis gallinae* present.

L. sylviarum in small numbers. The bird died of bronchitis.

(4) Feb. 27, White Plymouth Rock pullet.

Bird too fat.

Heart enlarged.

Tracheae very haemorrhagic and blocked with bloody exudate.

Liver—a few lesions of *Histomonas meleagridis*.

Caecum—a few *Heterakis gallinae*.

Duodenum slightly inflamed.

A few *L. sylviarum* present. The bird died of bronchitis.

(5) Feb. 25, White Plymouth Rock pullet.

Findings as for (3), but *L. sylviarum* infestation heavy (duration unknown). Death probably due to bronchitis.

(6) Feb. 27, White Plymouth Rock pullet.

Findings similar to (4). *L. sylviarum* infestation heavy (duration unknown). Death due to bronchitis.

(7) Feb. 27, White Plymouth Rock pullet.

Findings similar to (4) with a similarly light infestation of *L. sylviarum*. Death due to bronchitis.

(8) March 8, White Plymouth Rock pullet.

Bird emaciated.

Duodenum very haemorrhagic.

Very heavy infestation of *L. sylviarum* (duration unknown). Death may have been due to mites, or to the condition of the duodenum (cause unknown), or both.

(9) April 2, Rhode Island Red cock.

Right kidney greatly enlarged by tumorous growth (as large as a tennis ball), and duodenum highly inflamed. Heavy infestation of *L. sylviarum*. Death probably due to the other conditions.

(10) July 18, Barred Plymouth Rock cockerel.

Infested with *Capillaria* spp.

Infestation of *L. sylviarum* only fairly heavy. Death probably not due to mites.

(11) July 22, White Plymouth Rock cockerel.

Infested with *Capillaria* spp.

Infestation of *L. sylviarum* very heavy. Death might be due to this, or to *Capillaria* spp., or both.

(12) Aug. 15, Barred Plymouth Rock cockerel.

Tracheae blocked with mucus—bronchial trouble from March 7.

Infestation of *L. sylviarum* very heavy from March 26. Death probably due to the bronchial trouble, as the bird was sickly before infestation.

(13) Oct. 21, Barred Plymouth Rock cockerel.

Ascaridea galli present but *L. sylviarum* infestation also very heavy from June 15.

Death might be due to either or both *L. sylviarum* and *A. galli*.

This bird was outside from Sept. 7 to Oct. 1, but the very heavy infestation remained the same.

Of the above birds, Nos. 9 to 13 had been artificially infested since March 7.

Observations on Live Birds

Records were kept of the weight, egg production, and infestation of 29 pullets from Feb. 12, 1936, to March 7. Unfortunately, the fowls were also infested with *Capillaria* spp. and infected with bronchitis. Many birds died about the middle of March.

Since so many factors, such as heredity, egg production and general health, are involved besides mite infestation, it is difficult to draw any conclusions from these observations. Therefore, the writer cannot support at present those authors who state that this mite causes great economic loss.

Birds which are in poor health owing to internal parasites, disease, heavy egg production, etc., tend to fall an easy prey to ectoparasites, since they become listless and consequently uncleanly in their habits. Thus the writer feels that mite infestation may be a secondary condition and not the primary cause of lack of condition or ill health.

B. Man

Riley and Johannsen (45) cite an instance of *L. sylviarum* attacking man and causing pruritis. This mite readily leaves infested birds, if the latter

are handled, and goes on to man, as already noted. It has been the writer's experience that this mite readily attacks man and bites soft-skinned parts of the body, causing intense itching.

Sambon (47) suggests that *L. sylviarum* can convey "chicken pox", caused by the filterable virus *Epithelioma contagiosum*, from birds to man. The better name for this disease is "fowl pox", since "chicken pox" is the name usually given to an acute contagious disease (principally of young children) caused by *Varicella* sp. and not infective to poultry. There seems to be no evidence to prove that man may become infected by "fowl pox" caused by *Epithelioma contagiosum*.

Since *L. bursa* and *L. sylviarum* are so closely related, the following reports are of interest. There are records of *L. bursa* biting humans (Cilento (13)) in an area in Australia where a disease occurs which closely resembles the mite-borne river fever of Japan. From other parts of Australia, from Zanzibar, Africa, India, and China, attacks of *L. bursa* on man are reported (Hirst (22, 23, 25)), (Roberts (46)).

Monteiro (40) failed to infect guinea pigs with *Rickettsia brasiliensis* sp.n., by injecting, peritoneally, some crushed specimens of *L. bursa*. Hirst (22) reports one specimen from a lizard, while Roberts (46) says that *L. bursa* attacks any animal after birds have left the nest. In 1935, specimens of *L. sylviarum* were taken from a Labrador collared lemming kept in the Institute of Parasitology at Macdonald College. There is a possibility that *Rickettsia* bodies may be transmitted by *L. sylviarum* and *L. bursa*.

VII. Control

Historical

Wood (59) was the first to report successful control of *L. sylviarum*. He recommends the following procedure.

Clean the poultry plant thoroughly and spray it with carbolineum. In warm weather dip fowls in a mixture of 1 gal. water, 1 oz. soap, and 2 oz. sulphur. (This method was also successful with *Cnemidocoptes gallinae* Raillet.)

In cold weather, dust fowls with sulphur. (Fowls so treated suffered no ill results even when sent out into rain. Half-grown chicks and mother hens were treated similarly. With baby chicks, the brood coops were cleaned and dusted with sulphur.)

Destroy nests of the European sparrow.

Wood also had success by dusting with pyrethrum or dipping in a mixture of 1 gal. water, $\frac{1}{3}$ oz. soap, and 1 teaspoonful of nicotine sulphate. He found that mercuric ointment greatly reduced the numbers but did not give 100% control. A lime-sulphur solution effectively killed the mites but broke down the feathers very badly.

Troop (51) reported "quite" successful results with sulphur dusting and found that nitro-benzol fumigation controlled mites but tainted unlaid eggs.

In the control of *L. sylviarum*, Bishopp and Wood (7), Kaupp (30), Bushnell and Brandly (9), and Maw (36) all recommend sulphur (dusting or dipping) with carbolineum spraying. However, Cleveland* (15) did not get complete control by such methods and adds that birds brought from outside or returning from shows should be isolated and inspected. If infested, they should remain in isolation until successfully treated.

Bishopp (5) and Caesar (10) replace carbolineum by anthracene oil, a good grade of kerosene oil, or creosote oil. Caesar adds one part of sodium fluoride to every four parts of sulphur to kill lice. Payne*(43) also uses sodium fluoride for control of lice.

Payne* (42, 43) found that the greasing of small chicks with lard and vaseline was effective. For other birds, nicotine sulphate, applied 20 min. before roosting time every few days, was successful at any season and eliminated handling of the fowls.

Cutwright* (16) achieved temporary control by giving roosts a single treatment of nicotine sulphate and dusting nests with sulphur. Dipping birds and painting and spraying houses with a creosote and kerosene mixture failed to give control.

Bushnell and Brandly (9) state that dipping fowls in ½% zenoleum gives control, but best results are obtained by three treatments of nicotine sulphate on the roosts. Nicotine sulphate is also recommended by Bishopp and Wood (7), Jull (29), and Taylor (50).

Bishopp and Wagner (6) stated that a single treatment of nicotine sulphate is not sufficient, and the number of treatments depends upon the extent of infestation and the supplemental measures employed. After a reading of this paper, Cutwright reported verbally that in 1929 there was a severe infestation of 2,500 birds on the Experimental Farm at Wooster, Ohio, and one application of nicotine on the roosts killed all mites in 24 hr. No reinfestation occurred over a period of two years' observation.

Maw (36) states that nicotine sulphate is not satisfactory in all cases and that too much is detrimental to birds. An oil spray in the plumage of birds, as used in stables or on cattle, has given good results.

Whitehead and Maw (58) recommend a mixture of one part naphthalene flakes to two parts vaseline as giving 100% control on birds when applied around the tail and vent, or on the perches. Repeated tests were made, all of which were satisfactory. A mixture in the same proportions of "dichloricide" (paradichlorobenzene) and vaseline gave satisfactory results, but the "dichloricide" costs more and tends to liquefy the vaseline. Nicotine sulphate was used on perches, but comparative tests in the laboratory indicated that its action is slower than that of naphthalene or dichloricide. Naphthalene and vaseline also controlled lice, but the fumes were more lethal to mites.

Maw (38), in laboratory tests on mites, reported on various commercial tar oil preparations, most of which were highly satisfactory.

Naphthalene and Dichloricide Salves

A solid dichloricide salve may be made for summer use by mixing some paraffin wax with vaseline. The dichloricide is finely powdered and stirred into the molten wax-paraffin mixture and allowed to cool. This salve can be made of any desired consistency by varying the proportions of paraffin wax to vaseline, and will not melt at summer temperatures.

The roosts of two pens were smeared with naphthalene and dichloricide salves respectively. About a dozen male birds, infested with *L. sylviarum*, were placed in each pen. Paper was placed beneath the roosts. One day later, some inactive mites were found on the paper. However, there was scarcely any appreciable decrease in the infestation on the birds in either pen, although most of the mites had migrated from the tail feathers to beneath the wings and along the back. No further effects were noted during the next week, and the mites again returned to infest the anal feathers. Naphthalene and dichloricide, in mixtures of twice the strength recommended by Maw (38) for application to the bird's body are relatively ineffective when used on the roosts.

Nicotine Sulphate on Bird's Body

On March 12, 1936, 12 White Plymouth Rock laying pullets were treated with nicotine sulphate by applying drops to the anal feathers, under each leg, under each wing, and on the neck. On March 15, these birds were clean, while untreated infested birds (controls) in the pen still carried mites. No injury was done to the birds, but this method of control involves handling.

Comparison of "Dichloricide", Naphthalene, and Nicotine Sulphate

Six male and three female birds, heavily infested with mites, were placed individually in coops. The latter were divided into three groups, and had the roosts smeared with either naphthalene salve, dichloricide salve, or nicotine sulphate liquid (Black Leaf 40). The birds were examined daily for seven days. At that time, the infestation was unchanged in the first two groups, as were three controls in untreated coops. But the three birds treated with nicotine sulphate were clean, the female being free of mites from the second day, the males from the fourth and fifth days, respectively. Attempts to reinfest these birds on the sixth and seventh days were unsuccessful.

Eggs laid by hens in the first two groups were slightly tainted by the chemicals used in the salves.

Further Tests with Nicotine Sulphate

The roosts in a pen housing 55 infested birds (White Plymouth Rock laying pullets) were treated with $1\frac{1}{4}$ oz. nicotine sulphate. This was spread over the entire 60 ft. of roost space. The following evening a further $\frac{3}{4}$ oz. was applied to roosts and edges of nest boxes. All birds found off the roosts were put on them. Five of the birds had been placed in coops above the nest

boxes, as controls, on the first day. Five days later, these controls carried their original infestation, while the remaining 50 birds were free from mites. The controls were freed by smearing drops of the liquid on tail, thighs, wings, and head. They showed no ill effects.

All of 57 Barred Plymouth Rock laying pullets, treated in the same way, were free from mites after four days. Four controls remained infested as on the first day. They were treated as above.

Nicotine sulphate was used in several other infestations, and in each the birds were completely freed of mites by a single application. One may conclude, therefore, that nicotine sulphate, when applied under the above conditions, will control the northern fowl mite, *Liponyssus sylviarum*, C. and F. A single application of 2 oz. to every 50 birds is sufficient. The cost is about ½c. per bird.

Recommendations

Infested birds should be isolated from all others and should not have the same attendant as the clean birds.

In the infested pens, during frosty weather, the roosts, dropping boards and nest boxes should be scraped clean; and, about 20 min. before the birds go to roost, the roosts, roost supports and lower front edge of each nest box should be smeared with nicotine sulphate. A bird entering a nest box comes in contact with the nicotine sulphate on the front edge and is soon rid of mites. With a small brush apply about 2 oz. for every 50 birds present and treat every infested pen on the same evening, making sure, if possible, that every bird goes to roost.

Every bird arriving from another farm, plant, show or other place, should be isolated, examined for mites, treated (if infested) and kept in isolation until completely rid of the pest.

Single birds may be hand-treated with a few drops of nicotine sulphate smeared around vent, under thighs and wings, and on the neck.

Make a practice of treating all birds as soon as they enter winter quarters in the fall, since they may have picked up infestation from wild birds; and besides, this treatment is effective against lice. Prevent wild birds from nesting near poultry houses.

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